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STRUCTURE-ANTIMICROBIAL ACTIVITY RELATIONSHIP FOR A NEW CLASS OF ANTIMICROBIALS, SILANOLS, IN COMPARISON TO ALCHOLOS AND PHENOLS

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14. ABSTRACT

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We have recently discovered a new class of silicon based antimicrobials called silanols (R(CH3)2SiOH). The antimicrobial activity of the silanols was at least twice as strong as their analogous alcohols. The silanols are prepared from the hydrolysis of chlorosilanes. The silanols can be prepared by low cost processes. Silanols degrade into the environmentally benign species of silica, CO2 and H2O instead of accumulating in the environment.

Understanding the mechanisms of the antimicrobial action is critical for the development of antimicrobials with improved antimicrobial effects. A structural XIV dependency of the antimicrobial activity was investigated with four bacteria, *Escherichia coli*, *Staphylococcus aureus*, *Psudomanos aeruginosa*, and (Continued on page ii.)

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STRUCTURE-ANTIMICROBIAL ACTIVITY RELATIONSHIP FOR A NEW CLASS OF ANTIMICROBIALS, SILANOLS, IN COMPARISON TO ALCOHOLS AND PHENOLS

By

YUN MI KIM

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
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2006

This work is dedicated to my parent, my father, Lic Nam Kim, and my mother, Jung Le Son, in South Korea.

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By

Yun Mi Kim

August 2006

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CHAPTER 1
INTRODUCTION

1.1 Economic and Health Impact on Microbial Infections

Recently, a significant amount of attention has been directed toward development of novel classes of biocides because of the potential for microbial contamination, infection risks to military personnel and the general population, as well as growth of bacterial resistance to existing biocides. The economic and health effect caused by microbial contamination has been enormous. The Centers for Disease Control (CDC) estimated that foodborne diseases cause approximately 76 million illnesses, 325,000 hospitalizations, and 5000 deaths in the United States each year (Mead, 1999). The Economic Research Service (ERS) assessed the cost from five main bacterial foodborne pathogens such as *Salmonella* (nontyphoidal), *Escherichia coli* O157 at $6.9 billion in 2000 (Frenzen, 2004). The CDC also reported that 900 to 1,000 people lose their life each year due to microbial illnesses from U.S. drinking water (Gelt, 1998). Worldwide, more than 5 million people die each year, about 2.3 billion people suffer, and 6,000 people die daily as a result of waterborne diseases (Cloete, 2004).

Common diseases or symptoms caused by the four bacteria strains evaluated in this study are reported as follows. *Escherichia coli*, a Gram-negative bacterium, normally causes diarrhea, fever and abdominal cramps when a person eats contaminated food or drinks contaminated water. Surgical wound infections and meningitis in newborn due to *Escherichia coli* infection has been reported as well (Sussman, 1997). *Pseudomonas aeruginosa* is an opportunistic Gram-negative bacterium that usually causes problems in

humans who have weakened immune systems. It causes urinary tract infections, respiratory system infections, soft tissue infections, bone and joint infections, and gastrointestinal infections. *Pseudomonas aeruginosa* infection is a serious problem for patients with a weakened immune system due to cancer or AIDS, and for patients who have suffered severe burns (Fick, 1993). *Staphylococcus aureus*, a Gram-positive bacterium, is one of the main causes of pus and toxic food poisoning. It is the most common cause of infection in surgical wounds (Ayliffe, 2001). *Enterococcus faecalis* is an opportunistic Gram-positive bacterium inhabiting the alimentary canals of humans, but it becomes life-threatening to patients in hospital because it grows fast on wounds and burns. It can cause bladder infections, prostate infections, and, more rarely, heart and nervous system infections (Ayliffe, 2001).

Antimicrobial resistance has become a major public health concern. In hospitals, roughly 70% of bacterial pathogens are resistant to at least one antibiotic. For example, *Staphylococcus aureus* has become resistant to many commonly used antibiotics such as penicillin or methicillin. The misuse of antibiotics is known to be a main cause for the development multiple drug-resistant bacteria (Harrison, 1998). The bacterial resistance is developed through genetic processes of mutation and selection, the ability to exchange genes, and the fast growth rate of the resistant mutant (Guillemot et al., 2002). Bacteria may be inherently resistant to an antibiotic as well. For example, Gram-negative bacteria have an outer membrane that works as a permeability barrier against antibiotics (Atlas, 1984). Hence, new antimicrobial agents for which bacterial resistances have not been developed can be one solution for treating resistant bacteria as well as emerging new bacteria disease.

1.2 Current Disinfection Technologies in Comparison with Our New Antimicrobial Agents, Silanols

Various types of disinfection techniques are used to inhibit or destroy selective bacteria or other microorganisms. The disinfection techniques are mainly divided into two methods; physical methods include heat, cold, radiation, and hydrostatic pressure and chemical methods include chemical agents (Russell, 2001). The selection of the methods depends upon the contaminated substrates or target areas and the resistance of the microorganism. If contaminated substrates are vulnerable to heat or radiation, one needs to consider using alternative methods such as chemical agents. A combination of heat and pressure such as an autoclave system destroys most of microorganism including spores. However, this method has limitations in regard to space and availability. When a building, an office or a house is infected, the autoclave method is not appropriate. The radiation treatment is limited to surface disinfection. The use of chemical agents is the most common method for inactivation of microorganisms because it can be portable, economical, and can be applied to a variety of infected objects.

Chemical agents can be classified into two groups on the basis of their activity against microbes such as bacteria and spores. The first group is the so called bactericide or sporistatic (not sporicidal), such as alcohols, phenols, quaternary ammonium compounds and biguanides. The second group is sporicidal, such as peracetic acid (PAA), glutaraldehyde, chlorine-releasing agents, iodine-releasing agents, ethylene oxide and hydrogen peroxide (HP). A high concentration of the agents is normally required to achieve the sporicidal effects due to the highest resistance of spores (McDonnell and Russell, 1999). All terms with the suffix –cide imply an ability to kill, i.e., bactericide, sporicide, and biocide. Biocide is a general term describing a chemical agent that

inactivates microorganisms. The suffix –static is to describe an ability to prevent microbial growth without killing the microorganism (McDonnell and Russell, 1999).

A new generation of biocides is designed to be environmentally friendly, easy to apply using various delivery systems, and have strong activities toward a broad range of microbes. We have recently discovered a new and unexpected class of powerful biocides based upon compounds derived through simple chemistry from silicone intermediates, “silicon alcohols”called silanols (R'R"R'"SiOH). Silanols can inactivate microorganisms through various delivery systems such as a neat liquid phase, vapor phase due to their high volatility, and as bound antimicrobial agents. R'R"R'"SiO can be bound to hydroxyl containing substrates such as fabrics, glass, and wood through Si-O-C linkages (Isquith et al., 1972; Matsuo et al., 2005). Silanols released through hydrolysis from substrates can prevent microbial growth or inactivate microbes. The environmental fate of silicones has been recently reviewed (Graiver et al., 2003). The hydrolysis of polydimethylsiloxane elastomer occurs in aqueous environments to generate cyclic volatile methyl siloxane such as octamethylcyclotetrasiloxane, and further degrades into dimethylsilanediol and trimethylsilanol, ultimately oxidizing to environmentally benign silica, carbon dioxide and water in the environment. Graiver suggested that the silicone compounds are environmentally friendly materials since the degradation process occurs in a short period of time and generates the benign materials.

Antimicrobial agents commonly used, such as alcohols, phenolic compounds, and quaternary ammonium compounds (QACs), can be comparable to silanols in terms of kill mechanisms and biocidal activities. Alcohols including ethanol, isopropanol, and n-propanol are the most widely used disinfectants. Alcohol treatments have been effective

against bacteria and fungi but not for spores. The specific modes of action of the alcohols are not completely known, but it is generally believed that alcohols cause membrane damage (Dombek and Ingram, 1984; Lucchini et al., 1993; Lucchini et al., 1990) through an increase of lipid solubility and rapid denaturation of proteins which interferes with the cells metabolism and lysis (Pethica, 1958). Phenolic-type antimicrobial agents have long been used for their disinfectant or preservative properties. Various types of phenol derivatives such as alkylated phenol, halogenated-alkyl phenol, and phenyl-phenol have been developed to make the materials more effective, less toxic, and less irritating than phenols. Phenolic agents are bactericide, fungicide, and virucide for the hydrophobic virus (McDonnell and Russell, 1999). Denyer (1990) reported that the primary target site of phenolic agents was the cytoplasmic membrane. The damage to the cytoplasmic membrane causes the loss of the membrane's ability as a permeability barrier and subsequently leads to a loss of structural integrity and a leakage of intracellular material. Quaternary ammonium compounds (QACs) are surface active agents (surfactants). They have two regions in their molecular structures, one a hydrocarbon, water-repellent hydrophobic group and the other a water-attracting hydrophilic or polar group. Cationic types are the most useful antiseptics and disinfectants. However, they are not sporicidal. Quaternary ammonium compounds are known as membrane active agents with distruption of the cytoplasmic membrane of bacteria. The mode of action of cationic agents was proposed by Salton (1968). Adsorption and penetration of the agent into the cell wall occur followed by interaction with the lipid or protein in membrane that can lead to membrane disorganization. Leakage of low molecular weight components takes place as a result of the damage of the cytoplasm membrane (Salton, 1968).

Silanols, alcohols, and phenols have a similar chemical structure consisting of hydrophilic hydroxyl group and hydrophobic organic groups. It is reasonable to predict that the antimicrobial mode of actions of silanols may resemble those of alcohols and phenols. For many antimicrobial agents, antimicrobial actions are initiated by the interactions of the biocides with the cell wall membrane of the microorganisms. The agents, then, penetrate into the cell and finally act at the target sites (McDonnell and Russell, 1999). In this study we suggested that the hydrophobic interaction occurring between the organic group of silanols and the hydrophobic regions of membranes causes disorganization of the membrane. Ultimately the membrane loses the ability to regulate the passage of substances followed by a loss of structural integrity which will lead to death of the microbes. In our studies, the membrane damage of the bacteria after silanols treatment was primarily detected by an electron microscopy technique and a fluorescent dye method.

1.3 Study of Quantitative Structure-Activity Relationship (QSAR)

Investigation of the relationship between chemical structure and the activity of chemical compounds helps in understanding the mechanism of bioactivity and provides a tool to predict the activity of new compounds based on knowledge of the structural parameters. Quantitative structure-activity relationship (QSAR) methods have been utilized for estimating a potential toxicity of organic compounds and development of drugs (Hansch et al., 1995; Lill et al., 2005; Yen et al., 2005). A method for correlating biological activity with chemical structure was proposed by Hansch and Fujita in 1964. A general model for biological quantitative structure-activity relation was derived from a linear free energy-related approach, later called Hansch analysis. All parameters used in Hansch analysis were linear free energy-related values derived from rates or equilibrium

constants. The QSAR method was significantly improved by the combination of different physicochemical parameters such as the electronic or the hydrophobic property in a linear additive manner as shown in Equation 1-1. Hansch and Fujita derived a correlation model Equation 1-2 based on the linear free energy approach using physicochemical parameters (Hansch and Fujita, 1964; Hansch et al., 1995). Log 1/C is the logarithm of the inverse molar dose that produces a certain biological response, log P is the logarithm of the n-octanol/water partition coefficient as a measure of the hydrophobicity, and the Hammett constant σ is an electronic property. The parabolic Equation 1-3 was fit over a broad range of hydrophilic to lipophilic molecules. Those molecules could not cross the lipophilic or hydrophilic barriers of the membranes, and showed reduced bioactivity (Kubinyi, 1993).

$$\log(1/C) = (\text{steric}) + (\text{electronic}) + (\text{hydrophobic}) + \text{--- etc.} \quad (1\text{-}1)$$

$$\log 1/C = a \log P + b\sigma + \text{Const.} \quad (1\text{-}2)$$

$$\log 1/C = a (\log P)2 + b \log P + c\sigma + \text{Const.} \quad (1\text{-}3)$$

Regression analysis is a statistical method that can correlate independent variables, e.g., physicochemical parameters, with dependent variables, e.g., biological responses. This method is used to describe the relationship precisely by means of an equation that has a predictive value (Kubinyi, 1993). The multiple regression method was used in our study to correlate two independent variables with a dependent variable.

There have been a number of studies showing antimicrobial-structural dependence by evaluating a homologous series of aliphatic alcohols, alkylated phenol derivatives, and quaternary ammonimum compounds (Daoud et al., 1983; Klarmann, 1933; Kubo et al., 1995; Lien et al., 1968; Suter, 1941; Tanner, 1943). The relationship between the

hydrophobicity and antimicrobial activity of a homologous series of agents was mainly investigated. Tanner et al. (1943) examined the antimicrobial activity of aliphatic alcohols containing from 1 to 11 carbon atoms by employing nine different strains of bacteria. These authors showed that bioactivity increased as the alkyl chain length increased from methyl to pentyl, then decreased through normal hexyl, heptyl and octyl alcohols as a result of a decrease in water solubility. A study of the branching effect of the alcohols demonstrated a decrease in germicidal action from the primary to iso- to secondary to tertiary arrangement of the carbon chain (Tanner, 1943). In the case of phenols, the bactericidal actions of alkyl substituted phenol (Suter, 1941) and the normal alkyl derivatives of p-chlorophenols (Klarmann, 1932) were examined against Gram-negative and Gram-positive bacteria. These authors also reported that an increase of the alkyl chain length led to an increase of antimicrobial activity as well as observation of a fall-off of the activity. The fall off points varied with tested microorganisms. Hansch and Lien finally summarized the structure-activity relationship of antimicrobial agents as equations employing physicochemical properties (Lien et al., 1968) based on a method proposed by Hansch and Fujita in 1964. They observed that the correlations varied with the type bacteria and the type of antimicrobial agents. These authors suggested that the lipophilic property of the molecule was the most important factor for the antimicrobial activities of the compounds with a relatively minor contribution from electronic properties. Daoud et al. (1983) showed a parabolic relationship between the partition coefficient and antimicrobial activity by testing a homologous series of alkyldimethylbenzyl ammonium chloride, and quaternary ammonium salt compounds.

Etoh et al. (1994) also performed a quantitative analysis of the antimicrobial activity using the para-substituted alkylphenols against *Trychophyton mentagrophytes*.

The QSARs previously reported between the physicochemical properties and the antimicrobial activity were mainly established evaluating a homologous series of agents such as alcohols or phenol derivatives separately. In this study the structural dependence of antimicrobial activity was conducted against four bacteria, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomanos aeruginosa*, and *Enterococcus faecalis*. Silanols, $R(CH_3)_2SiOH$, a novel class of antimicrobial agents, alcohols with structures analogous to the silanols, $R(CH_3)_2COH$, and substituted phenols were evaluated as a single group of chemical compounds. The minimum lethal concentrations (MLC) defined as the concentration required for a 7-log reduction within 1 hour exposure time were used to measure the antimicrobial activity. The octanol/water partition coefficients (log P) and the H-bond acidities ($\Delta\nu$) measured as the shift in frequency of OH stretching band between free OH and hydrogen bonded OH to diethyl ether oxygen by infrared spectroscopy were utilized as the structural parameter. The goal of this study is to understand the relationship between the structural parameters and the antimicrobial activity of silanols, alcohols and, phenols, ultimately providing a tool to predict the biocidal properties of new compounds of silanols and carbinols through quantifying structure-activity relationships developed in this study.

CHAPTER 2
SYNTHESIS AND CHARACTERIZATION OF SILANOLS, ALCOHOLS, AND PHENOLS

2.1 Introduction

Silanols can be prepared by the hydrolysis of organosilicon halides, amines, hydrides, alkoxides, and esters. Water alone can lead to a rapid hydrolysis for most of the types, while alkoxides and hydrides require either a base or an acid as a catalyst. Since the condensation of silanols to siloxanes decreases the purity of silanols it would be important to keep the system as neutral as possible and maintain the reaction temperature as low as possible (George et al., 1953; Hyde, 1953; Kantor, 1953; Sauer, 1944; Sommer et al., 1946). The chemical structure of tested silanols, $R(CH_3)_2SiOH$, is analogous to the organic alcohols, $R(CH_3)_2COH$, except the central atom is silicon. It might be expected that the silanols would have similar physicochemical properties to their analogous alcohols because silicon and carbon belong to the group IVA of the chemical periodic table. However, silanols have a greater hydrophobicity due to flexible molecular chains and lower group rotation energy barrier than the carbon bond (Owen, 1990). In addition silanols were found to have a greater acidity by electron back donation from oxygen through (p→d orbital)π bond (West and Baney, 1958, 1959) when compared to analogous organic alcohols even though silicon is more electropositive which would make silanols less acidic than analogous carbinols. The octanol-water partition coefficients for the hydrophobic property and the H-bond acidities for electronic properties of the silanols and alcohols were determined as well as those properties of

phenols. These two estimated physicochemical properties were used to prove our hypothesis that the silanols showed a stronger antimicrobial activity than that of analogous alcohols due to their higher hydrophobicity and H-bond acidity. This hypothesis was studied by establishing the relationship between antimicrobial activities of silanols, alcohols, and phenols and their physicochemical properties presented in Chapter 4.

2.2 Experiment

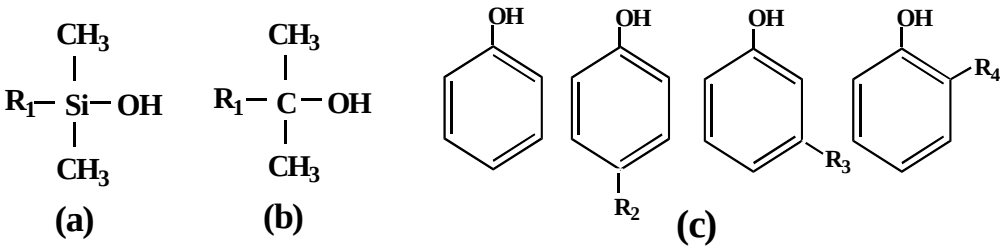
2.2.1 Materials

$R(CH_3)_2ClSi$, R=ethyl, n-propyl, n-butyl, phenyl, vinyl, benzyl, phenethyl, and acetoxytrimethylsilane were obtained from Gelest Inc. for preparation of the silanols. The analogous alcohols, $R(CH_3)_2COH$, and substituted phenols were obtained from Acros Organics. The substituents, R, of alcohols were methyl, ethyl, n-propyl, n-butyl, phenyl, vinyl, benzyl, and phenethyl. The substituents for phenol derivatives are 4-methyl, 4-ethyl, 4-propyl, 4-butyl, 4-pentyl, 4-hexyl, 3-chloro, and 2-phenyl.

2.2.2 Synthesis of Silanols

Silanols, $R(CH_3)_2SiOH$, were prepared by the hydrolysis of chlorosilane, $R(CH_3)_2ClSi$, and acetoxytrimethylsilane (George et al., 1953; Hyde, 1953; Kantor, 1953; Sauer, 1944; Sommer et al., 1946). We prepared two different flasks, called solution A and B respectively, and then the solution A and B were mixed for the hydrolysis reaction. Solution A was prepared by adding 13ml of chlorosilane or acetoxytrimethylsilane into 100ml of diethyl ether (anhydrous). Solution B was prepared with 75ml of deionized water, 25ml of ammonium hydroxide (29.4%) as a neutralizing agent, and 100ml of diethyl ether. Solution A was added into solution B and mixed at 20ºC for 15minutes for trimethylsilanol and ethyldimethylsilanol , 20-30 minutes for n-propyl-, n-butyl-, vinyl-,

and phenyldimethylsilanol, and 40-60 minutes for benzyl- and phenethyldimethylsilanol. The silanols dissolved in diethyl ether were separated from the water layer and washed with 100ml of deionized water. The diethyl ether layer containing the silanol was separated from the water layer, followed by an addition of sodium sulfate (anhydrous) to absorb any residual water. The diethyl ether solution was obtained by filtration of the absorbent. The silanols were obtained through evaporation of diethyl ether at 34 ºC.



R_1= 1. CH_3 2. C_2H_5 3. n-C_3H_7 4. n-C_4H_9 5. CH_2=CH 6. C_6H_5 7. $C_6H_5CH_2$ 8. $C_6H_5CH_2CH_2$

R_2= 1. CH_3 2. C_2H_5 3. n-C_3H_7 4. n-C_4H_9 5. n-C_5H_{11} 6. n-C_6H_{13}

R_3= Cl R_4= C_6H_5

Figure 2-1. Chemical structures (a) silanols (b) alcohols (c) phenols

2.2.3 Characterization of the Silanols, the Alcohols, and the Phenols

Characterization of the silanols and purities of silanols, alcohols, and phenols. Spectroscopic techniques such as Fourier Transform Infrared spectroscopy (FTIR) and H-1, Si-29 Nuclear Magnetic Resonance (NMR) were employed to confirm the synthesized silanols and their purities (Brook, 2000; Lipp, 1991). Attenuated total reflectance (ATR) technique for FTIR was utilized for the liquid silanol samples. The neat sample was placed on top of an ATR crystal (ZnSe) and subsequently the infrared spectrum acquired. For NMR spectroscopy 0.1ml of silanol and 0.6ml of NMR solvent Benzene-D_6 obtained from Cambridge Isotope Laboratories Inc. were mixed in a NMR tube. The 500MHz NMR spectrometer at the McKnight Brain Institute at the University of Florida was used for H-1 and Si-29 NMR experiment. The purities of the alcohols and the phenols, no less than 95%, were confirmed by Gas Chromatograph/ Mass spectrometer, Finnigan Trace

DSQ Single Quadrupole GC-MS with AS 3000 Autosampler, at the Department of Chemistry at the University of Florida.

Measurement of the H-bond acidity. The H-bond acidity of the silanols, the alcohols, and the phenols were determined by measuring Δν, defined as the shift in frequency of OH stretching band between free OH and hydrogen bonded OH to diethyl ether oxygen using infrared spectroscopy (West and Baney, 1959). The shift in frequency of the OH stretching band upon hydrogen bond formation proposed by Badger (1940)and Bauer (1937), showed a relation between the energy of a hydrogen bond and the frequencies of the OH bands. A linear enthalpy-frequency shift relationship was also shown by Sherry et al. (1972) and Ouvrard (2001). These studies suggested that the shift, Δν, should be a measure of the strength of the hydrogen bond. The strength of the hydrogen bond is based on the ability of the proton of the hydroxyl compound to associate with a proton acceptor site in the hydrogen bond base, diethyl ether. The shift is a measure of the relative proton donating ability of the OH-containing compounds because the proton accepting ability remained constant by employing the same base, diethyl ether.

Test solutions of the silanols, the alcohols, and the phenols were prepared at 0.04M in carbon tetrachloride respectively. The Lewis base, anhydrous diethyl ether, was prepared at 0.5M in carbon tetrachloride. 1ml of the base solution and 1ml of the silanols, alcohols, or phenols solution were mixed for the infrared spectroscopy study. The possibility of the self-association bands is negligible at the low concentration of 0.02M of the silanols, the alcohols, and the phenols (West and Baney, 1959). A transmission sampling technique using a NaCl window material was utilized for the infrared

spectroscopy measurements. Two OH bands in the 3800 -3200 cm^{-1} region were observed. A broad OH band was detected at lower frequencies, 3500-3200 cm^{-1}, due to hydrogen bonded OH to diethyl ether oxygen, whereas a sharp free OH band was observed at higher frequencies, 3800-3550 cm^{-1}. The difference in frequency between the two bands, $\Delta\nu$, measures the relative H-bonding acidity of the silanols, the alcohols, and phenols.

Calculation of the octanol-water partition coefficient. The lipophilic nature can be determined by partitioning a compound between an aqueous and a non-aqueous phase. The octanol-water partition coefficient (log P (o/w) is defined as the ratio of the concentration of a solute in a non-polar solvent (1-octanol) and the concentration of the same species in a polar solvent (water) under equilibrium conditions. The log P of the silanols, the alcohols, and the phenols were calculated by using the demo program, LogKow (KowWin), provided by Syracuse Research Corporation. The program estimates the log P by using the atom/fragment contribution (AFC) method (Meylan and Howard, 1995). The atom/fragment contribution approach proposed by Hansch et al. (1973, 1977) considers that a chemical structure is divided into fragments such as atom or larger functional groups. The values of each group are summed together with structural correction factors to estimate the log P. The AFC method was developed through multiple linear regressions of experimental log P values (Meylan and Howard, 1995). The first regression analysis was correlated with atom/fragment values without a correction factors. The second regression analysis was correlated with correction factors, and derived from the difference between the log P estimated from the first regression and the measured log P values. The log P of a compound was then estimated by simply summing

all atom/fragment values and correction factors contained in a structure. Examples of calculation of log P are displayed in Table 2-1.

Table 2-1. Examples of calculation of log P of t-butanol and trimethylsilanol using KowWin (LogKow) program.

KowWin(LogKow) Log P calculation

t- butanol
SMILES: OC(C)(C)C
CHEM
MOL FOR: C4H10O1
MOL WT: 74.12

TYPE	NUM	LOGKOW v1.66 FRAGMENT DESCRIPTION	COEFF	VALUE
Frag	3	-CH3 [aliphatic carbon]	0.5473	1.6419
Frag	1	-OH [hydroxyl, aliphatic attach]	-1.4086	-1.4086
Frag	1	-tert Carbon [3 or more carbon attach]	0.2676	0.2676
Const		Equation Constant		0.2290
		LogKow Estimated Log P		0.73

Trimethylsilanol
SMILES:O[Si](C)(C)C
CHEM:
MOL FOR : C3H10O1Si1
MOL WT: 90.20

TYPE	NUM	LOGKOW v1.66 FRAGMENT DESCRIPTION	COEFF	VALUE
Frag	3	-CH3 [aliphatic carbon]	0.5473	1.6419
Frag	1	-OH [hydroxyl, aliphatic attach]	-1.4086	-1.4086
Frag	1	-Si- [silicon, aromatic or oxygen attach]	0.6800	0.6800
Const		Equation Constant		0.2290
		LogKow Estimated Log P		1.14

2.3 Results and Discussion

2.3.1 Characterization of Synthesized Silanols

Infrared spectroscopy was used to confirm the relative purity of the synthesized silanols. The most characteristic band in the spectra of the organosilicon compounds is the symmetrical CH_3 deformation mode at 1260cm^{-1}. This band was observed as an

intense and sharp peak in the 1262±5cm^{-1} range. For the SiOH group, the spectrum depends on the degree of the OH group involved in the hydrogen bonding. In the case of free OH, the OH stretching vibration of SiOH absorbs at 3695cm^{-1} whereas hydrogen bonded OH to other silanols is found near 3300cm^{-1}. In our study, the band falls at 3300cm^{-1} implying that silanol molecules are hydrogen bonded to other silanols or residue of diethyl ether. These hydrogen-bonded absorbances were very broad and their exact positions are influenced by neighboring functional groups.

Disiloxanes are a by-product of condensated silanols and are present as an impurity. The siloxane absorption is usually the most intense band as well. The SiOSi group, asymmetric stretching bands, can be found near 1020-1090cm^{-1} as a single or doublelet strong, rather broad band. The SiOSi band was barely observed for the triethylsilanol due to its high purity, greater than 97% as seen in Figure 2-2.

For trimethylsilanol, NMR data showed that its purity was about 95%. The impurity, disiloxane, was identified as a Si-O-Si band around 1090cm^{-1} as shown in Figure 2-3. Other infrared spectra of the silanols are presented in Appendix A. The purities of the silanols were also determined using nuclear magnetic resonance (NMR) spectroscopy. Si-29 NMR was used to confirm the information obtained from H-1 NMR. The purities of the silanols were calculated from the peak integral ratio between Si on Si-OH and Si on Si-O-Si for the Si-29 NMR, and the peak integral ratio between proton on OH and protons on CH_3 for the H-1 NMR. The Si-29 isotope is the only naturally occurring NMR active silicon isotope. It has a spin quantum number I=-1/2 and a natural abundance of only about 4.7% (Brook, 2000). The problem of low abundance is a relative insensitivity of the nucleus. The majority of the chemical shifts of silicon-29 NMR occur

between +50 and -200 ppm. The chemical shifts of the silicon compounds are sensitive to neighbor effects in the chain structure. The exchange of a R substituent for oxygen leads to an upfield shift. It should be pointed out that the chemical shift of H-1 NMR of CH_2 of the triethylsilanol is located in upfield compared to that of CH_3, as shown in Figure 2-4, because silicon is electropositive relative to carbon, therefore protons from CH_2 is highly shielded (Silverstein and Webster, 1997). The Si-29 NMR confirmed that trimethylsilanol has a few percent of hexamethyldisiloxane as indicated by the two peaks in Figure 2-5. The small peak in upfield chemical shift corresponds to the disiloxane. The purities of the silanols measured by Si-29 and H-1 NMR are summarized in Table 2-2. Si-29 NMR showed a single peak when the purity was more than 97%. The impurities of the silanols were identified as the silanol condensation products, dialkyltetramethyldisiloxanes. NMR spectra for other silanols are displayed in Appendix A. The purities of phenyl-and phenethyldimethylsilanol synthesized were relatively low approximately 93±2 and 90±2% respectively. Their disiloxane impurities were clearly detected by Si-29 NMR as presented in Appendix A.

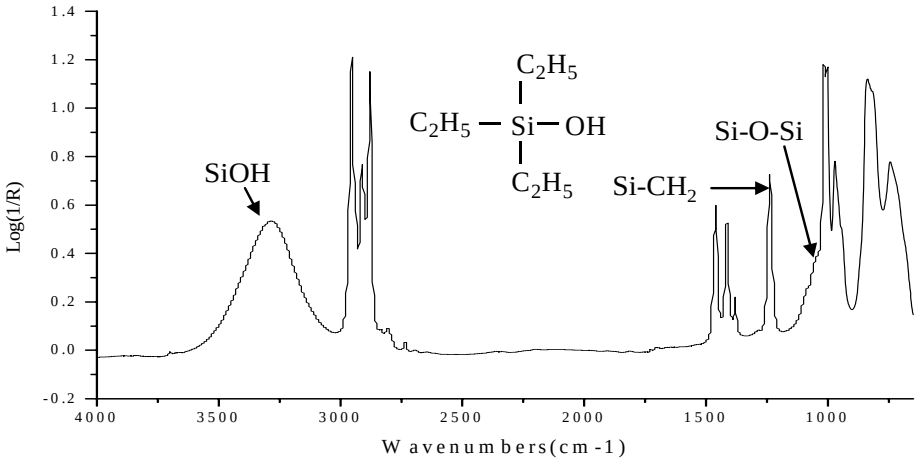


Figure 2-2. An infrared spectrum of triethylsilanol purchased by Gelest Inc.

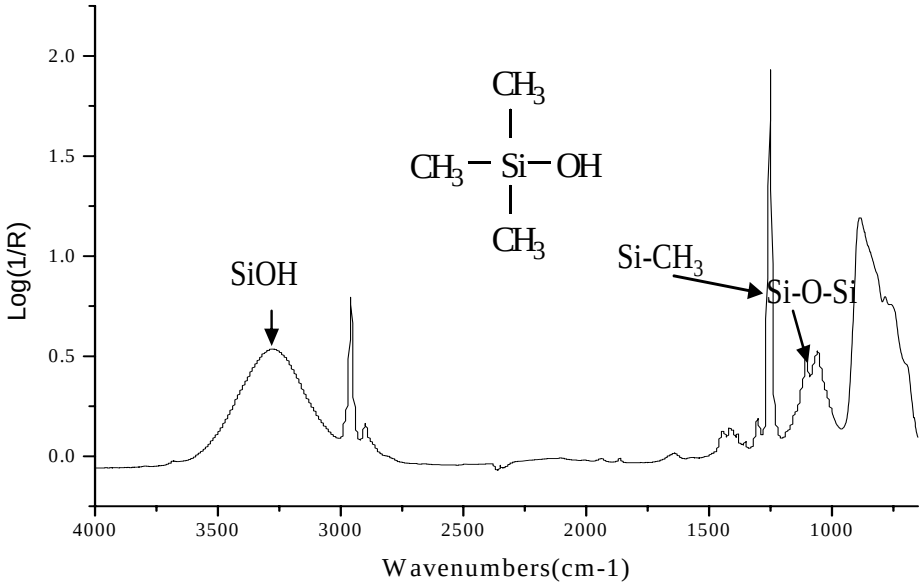


Figure 2-3. An infrared spectrum of trimethylsilanol

Table 2-2. Purities of alkyldimethylsilanol measured by H-1, and Si-29 NMR spectroscopy.

R	Purity of $R(CH_3)_2SiOH$	
	H-1 NMR (%)	Si-29 NMR (%)
CH_3	95±2	≥97
CH_2=CH	95±2	≥99
C_2H_5	96±2	≥99
n-C_3H_7	96±2	≥99
C_6H_5	93±2	93±2
n-C_4H_9	96±2	≥99
$C_6H_5CH_2$	96±2	≥99
$C_6H_5CH_2CH_2$	90±2	90±2
n-C_5H_{11}	96±2	≥99
n-C_6H_{13}	96±2	≥99
n-C_8H_{17}	96±2	-

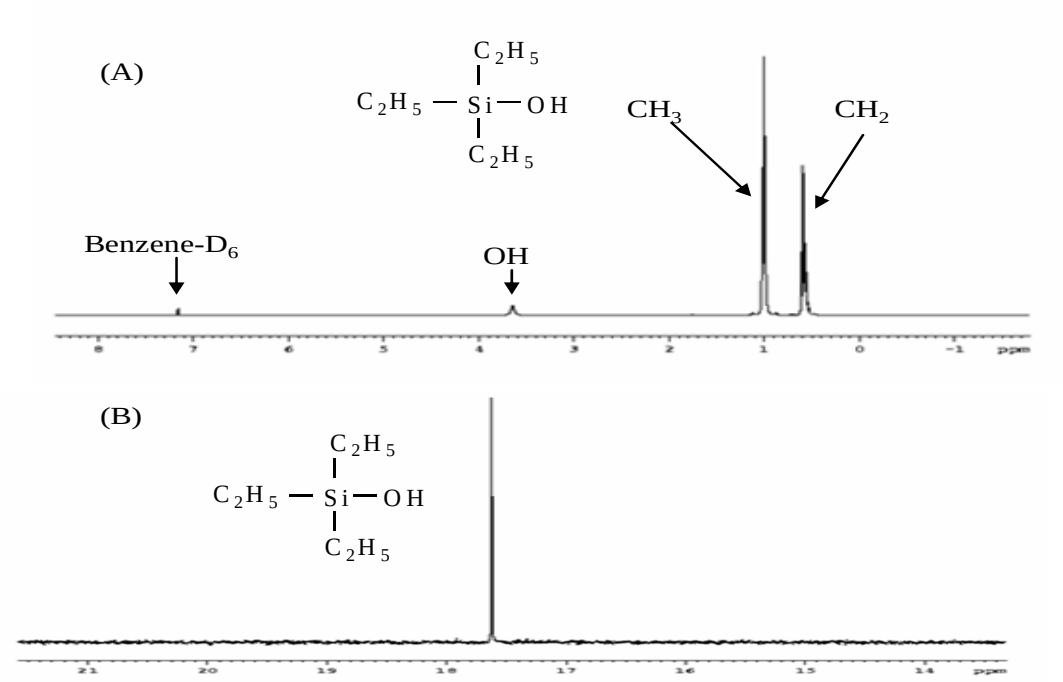


Figure 2-4. NMR spectra of triethylsilanol, A is H-1 NMR, B is Si-29 NMR in Benzene-D_6 solvent

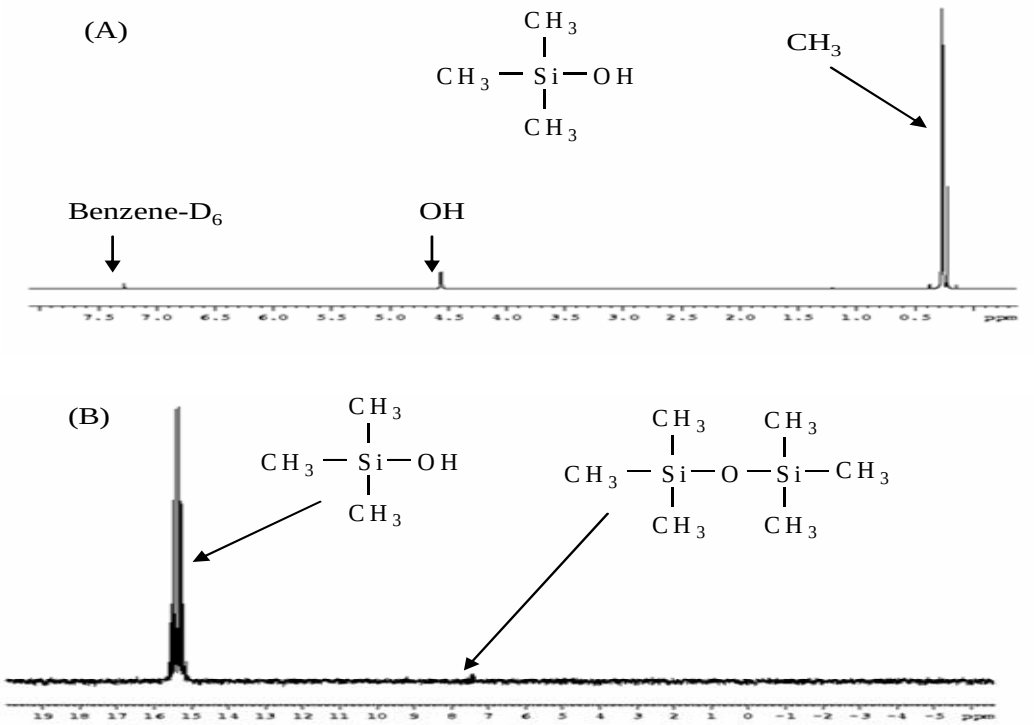


Figure 2-5. NMR spectra of trimethylsilanol, A is H-1 NMR, B is Si-29 NMR in Benzene-D_6 solvent.

2.3.2 The H-bond Acidity

The H-bond acidity of silanols, alcohols, and phenols measured by FTIR is the shift, Δν, a measure of the strength of the hydrogen bond, and summarized in Table 2-3 and 2-4. Two OH bands corresponding to silanols, alcohols, and phenols in the 3800 - 3200 cm^{-1} region were observed as shown in Figure 2-6, 2-7, and 2-8 respectively. The broad OH bands were detected at lower frequencies due to hydrogen bonded OH to the ether oxygen, whereas the sharp free OH bands were observed at higher frequencies. The measured H-bond acidities of silanols were almost two times higher than the analogous alcohols. The results were consistent with Dr. Baney's study (West and Baney, 1959). The hydrogen bonding acidity of several silanols and carbinols were compared, measured by the O-H stretching infrared band shifts (West and Baney, 1958, 1959). They reported that silanols produce the shift nearly twice as great as do the analogous alcohols, suggested that silanols are much more acidic than the corresponding alcohols. In the case of the silanols, the electron back donation through bond from p orbital of oxygen to vacant d orbital of silicon makes silanols more acidic than that of organic alcohols (Brook, 2000). In other words, the removal of electron density from oxygen makes the hydrogen more acidic. The H-bond acidities varied with substituents. Silanols, alcohols, and phenols containing aryl, vinyl, and chloro group exhibited a stronger acidity than those of alkyl groups. Relative acid strengths of alcohols, ROH, were estimated by Kuhn (1952) employing the shift in frequency. Kuhn showed that the acidity increases in the order of R=C_6H_5> CH_3> n-Bu> t-Bu. The H-bond acidity represents the electronic properties of substituents when the parent compound is fixed. If the electron attracting ability of the substiuent is greater the H-bond acidity is greater. Aryl, vinyl, or chloro

substituents are known to be electron withdrawing, whereas alkyl groups are electron supplying relative to methyl.

In the case of the silanols the broad H-bonded OH bands were found near 3450cm^{-1} and the free OH bands were detected at around 3690cm^{-1}. In comparison the alcohols have the broad H-bonded OH near 3480cm^{-1} and the free OH at 3610cm^{-1}. The substituted phenols 4-alkylphenols showed the H-bonded OH peak at 3340cm^{-1} and the free OH peak at 3610cm^{-1} while 3-chlorophenol displayed H-boned OH at 3287cm^{-1} and free OH at 3606cm^{-1}. 2-phenylphenol exhibited its H-boned OH at 3313cm^{-1} and free OH at 3564cm^{-1}. Most of the H-bonds formed were intermolecular hydrogen bonding between OH from silanols or alcohols and oxygen from Lewis base, diethyl ether due to the low concentration of silanols or alcohols. Benzyldimethylcarbinol, however, showed intramolecular H-bond peak, demonstrating two peaks of equal intensity (Baney, 1960) as shown in Figure 2-7.

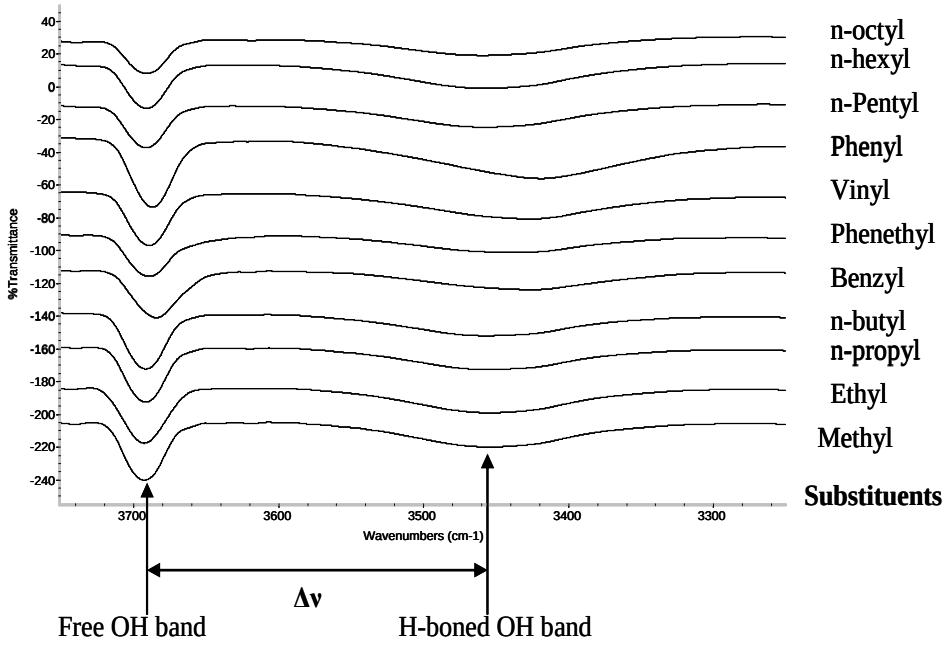


Figure 2-6. Infrared spectra for the H-bond acidities of the silanols($R(CH_3)_2SiOH$), X-axis is wavenumbers(cm^{-1}), Y-axis is % transmittance.

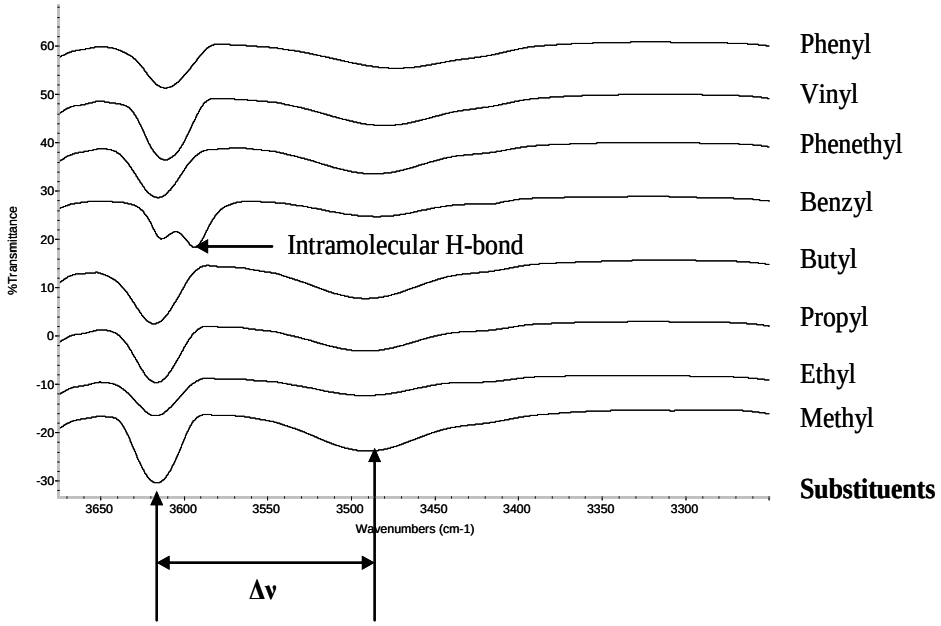


Figure 2-7. Infrared spectra for the H-bond acidities of the alcohols($R(CH_3)_2COH$), X-axis is wavenumbers(cm^{-1}), Y-axis is % transmittance.

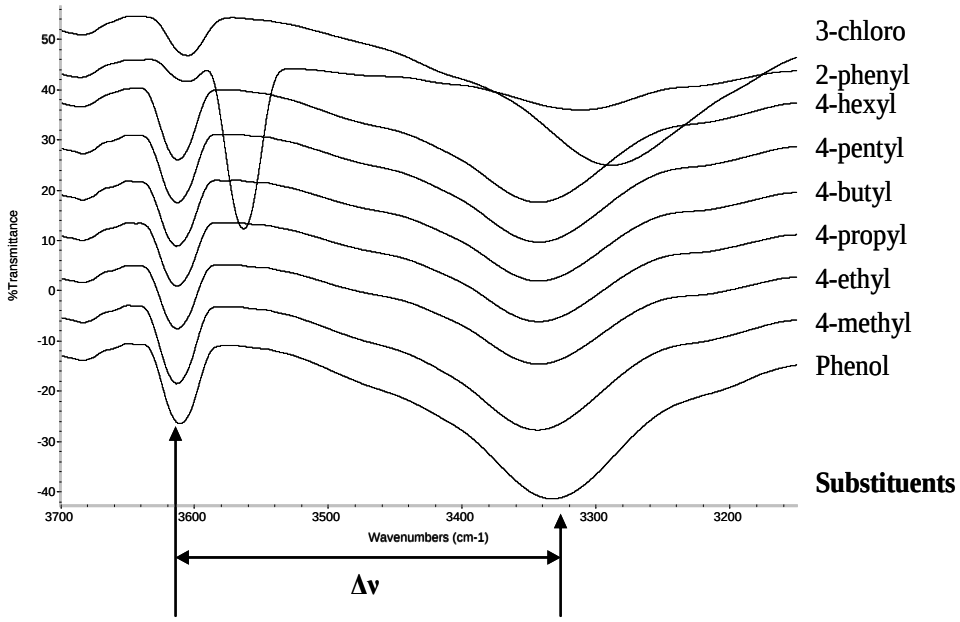


Figure 2-8. Infrared spectra for the H-bond acidities of substituted phenols, X-axis is wavenumbers(cm^{-1}), Y-axis is % transmittance.

2.3.3 The Partition Coefficient

The partition coefficients calculated by the atom/fragment contribution method (Meylan and Howard, 1995) are presented in Table 2-3 and 2-4. A gradual increment of the partition coefficient as the alkyl chain increase for silanols, alcohols, and phenols was

verified. The partition coefficient, log P, represents the degree of the compound's hydrophobicity. A higher hydrophobicity is a result of a decrease in free energy of an aqueous system. The free energy of the system decreases due to the increased association between water and longer alkyl substituent molecules occupying a larger space. The partition coefficients of the silanols were higher than the analogous alcohols as presented in Table 2-3. It is well known that silicon compounds including the silanols exhibit higher hydrophobic properties than analogous organic compounds due to flexible molecular chains and lower group rotation energy barrier than the carbon bond (Owen, 1990). Substituents on silicon element such as methyl or phenyl can rotate freely with a larger spatial occupation due to a lower energy barrier and longer chain length. The increased association between water and substituents from silanols contribute to lower the free energy. 4-pentylphenol and 4-hexylphenol exhibited one of the highest partition coefficients due to their long alkyl chain.

Table 2-3. H-bond acidity (Δν) and octanol-water partition coefficient (log P) of the silanols ($R(CH_3)_2SiOH$) and the alcohols ($R(CH_3)_2COH$).

R	Partition coefficient (log P)		H-bond acidity (Δν) in ether	
	$R(CH_3)_2SiOH$	$R(CH_3)_2COH$	$R(CH_3)_2SiOH$	$R(CH_3)_2COH$
CH_3	1.14	0.73	238	126
CH_2=CH	1.5	1.08	260	130
C_2H_5	1.63	1.22	237	125
n-C_3H_7	2.12	1.71	237	125
C_6H_5	2.36	1.95	267	137
n-C_4H_9	2.62	2.2	236	126
$C_6H_5CH_2$	2.85	2.44	257	129
$C_6H_5CH_2CH_2$	3.34	2.93	252	129
n-C_5H_{11}	3.11		234	
n-C_6H_{13}	3.6		235	
n-C_8H_{17}	4.58		232	

Table 2-4. H-bond acidity (Δν) and octanol-water partition coefficient (log P) of the substituted phenols.

Phenols	Partition coefficient (log P)	H-bond acidity (Δν) in ether
Phenol	1.51	278
4-methylphenol	2.06	270
4-ethylphenol	2.55	269
4-propylphenol	3.04	270
4-butylphenol	3.53	270
4-pentylphenol	4.02	269
4-hexylphenol	4.52	271
3-chlorophenol	2.16	318
2-phenylphenol	3.28	252

2.4 Conclusions

The silanols were prepared through the hydrolysis of chlorine derivative silanes. The synthesized silanols were confirmed by FTIR techniques and their purities were measured by H-1 and Si-29 NMR. The octanol-water partition coefficients were estimated by the LogKow (KowWin) program. The silanols were more hydrophobic than the analogous alcohols. The polar properties of the materials were estimated by measuring the H-bond acidity which is a measure of the hydrogen bond strength using infrared spectroscopy. The H-bond acidities of the silanols were approximately two times greater than those of analogous alcohols. The partition coefficient and the H-bond acidity of the phenols were also obtained in the same manner.

CHAPTER 3
ANTIMICROBIAL ACTIVITIES OF SILANOLS, ALCOHOLS AND PHENOLS

3.1 Introduction

Though organosilicon compounds are frequently claimed to be biologically inert, there is significant evidence that suggests their possible bioactivity. The discovery of the bioactivity of organosilicon compounds, silatranes, $RSi(OCH_2CH_2)_3N$, made by Voronkov changed the traditional thought of the biological inertness of silicon (Voronkov, 1988). Voronkov (1979) demonstrated the toxicity of silatranes, showing that 1-arylsilanetranes was highly toxic for warm-blooded animals. Toxicity of silatranes was affected by the nature of the substituents, R, in the structure. 1-Arylsilatranes were very toxic, whereas 1-alkyl, 1-vinyl-, and 1-ethinylsilatranes were practically non-toxic. Amines containing organosilicon compounds were tested as antimicrobial agents and insect-repellent by Lukevics (1978). Organosilicon amines, $RR'_2Si(CH_2)_3NR"R'"$, showed inhibition of the growth of some fungi such as *Candida albicans* and bacteria like *Staphylococcus aureus*. Their antimicrobial activities varied with substituents of the agent and microorganisms. The range of the minimum inhibitory concentrations was broad, ranging from 0.5 to 200µg/ml (Lukevics, 1978). Organopolysiloxanes are important materials for therapeutic applications because of their physicochemical properties and their inertness to biochemical processes. However, not all organopolysiloxanes are inert to biochemical processes. Bennett et al. (1972), Hobbs et al. (1972), and Palazzol.Rj et al. (1972) evaluated the toxicology of cyclosiloxanes containing at least one phenyl group and low molecular weight organosiloxanes, showed

bioactivities such as depression of male reproductive function or testicular atrophy in the rat and rabbit. The bioactivity correlated with high stereospecifity revealed that 2,6-cis-diphenyl-hexylmethyl-cyclotetrasiloxane was the most active compound, approximately 100 times more potent than the isomeric 2,6-trans-diphenyl-hexylmethyl-cyclotetrasiloxane for endocrinological activity in male and female rats (LeVier, 1978). Diphenylsilanediol was ascribed to anticonvulsant activity. In the case of seizures provoked by electroshock diphenylsilanediol has an ED50 of 25mg/kg after 2 hour whereas phenyldimethylsilanol has 118mg/kg (LeVier, 1978). ED50 is defined as an effective dose that produce desired effect in 50% of population. The anticonvulsant properties of the silicon compounds and their carbon analog were compared. Silicon compounds were more active, i.e. diphenylmethylsilanol has ED50 80mg/kg while diphenylmethylcarbinol has ED50 154mg/kg (LeVier, 1978). Recent environmental toxicology studies on fish showed that rainbow trout had 14-D LC50=10µg/L of octylmethylcyclotetrasiloxane (Sousa et al., 1995). Bennett et al. (1973) evaluated toxicology of trimethylsilanol, which is a potential end group hydrolysis product of polydimethylsiloxane by testing on mouse, rat, rabbit, and monkey models. These authors claimed that trimethylsilanol was about three times more potent than *t*-butanol. We report here a recent discovery of the strong antimicrobial effect of silanols against various bacteria (Kim et al., 2006) and fungus. Silanols, $R(CH_3)_2SiOH$, and their analogous alcohols, $R(CH_3)_2COH$, were tested to compare their antimicrobial activity against two Gram-negative bacteria and two Gram-positive bacteria. The antimicrobial activity of the substituted phenols was also collected under our experimental conditions.

The bacteria domain is divided into two groups based on the cells reaction to a staining procedure that was developed in 1888 by Christian Gram. Gram-positive bacteria retain the purple dye when stained by crystal violet, whereas, Gram-negative do not retain the purple dye. The cell structure is shown in Figure 3-1 and the bacterial chemical composition is different. Gram-positive bacteria have a thick and uniformly dense cell wall, consisting of 40-90% of peptidoglycan, teichoic acids, teichuronic acids, and sugar, while the Gram-negative bacteria have a thin and complex cell wall with an outer membrane (lipopolysaccharide, porin, and phospholipid) and 5-10% of peptidoglycan (Atlas, 1984; Barton, 2005).

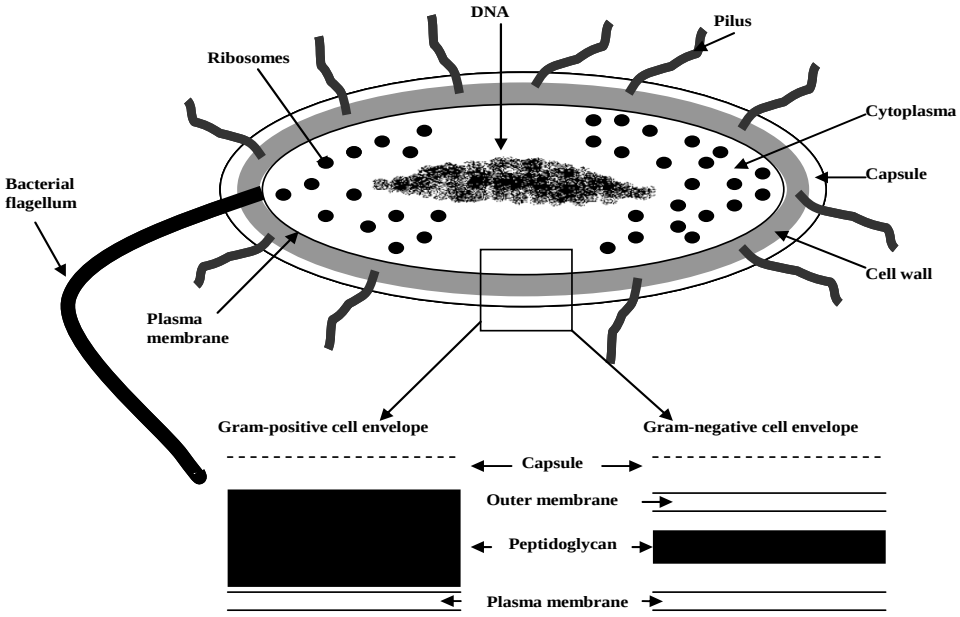


Figure 3-1. A general figure of bacterium and cell envelope of Gram-positive and Gram-negative bacteria.

The peptidoglycan holds the cell shape and prevents osmotic lysis, but not a permeability barrier. In contrast, the outer membrane of the Gram-negative bacterial cell is a permeability barrier meaning that molecules are selectively permeable dependent upon their size and lipophilicity. Both types of bacteria have a plasma membrane which

is a permeability barrier mainly composed of phospolipid and proteins (Atlas, 1984, 1986).

The minimum lethal concentrations (MLC) of tested antimicrobial agents were determined under a given experimental set-up of a 1 hour mixing time and a bacteria concentration of $2\text{-}6\times10^{8}$ cfu/ml. Since the MLC values vary according to exposure time, bacterial concentration, and bacterial strains experiments were performed to determine how the MLC values change depending upon the experimental conditions. Impurities of silanols are disiloxanes, condensation by-product of silanols. The antimicrobial effects of the silanols impurities were evaluated. Fungi are eukaryotic organisms unlike bacteria which are prokaryotes. Prokaryotes' genetic material, or DNA, is not enclosed in the nucleus, whereas eukaryotic organisms have DNA enclosed in a nucleus. Fungi cell wall is made of chitin, a polysaccharide, made out of units of acetylglucosamine (N-acetyl-D-glucos-2-amine) (Atlas, 1986). However, the cell walls of all bacteria contain a unique type of peptidoglycan called murein. It is possible that the different cell wall structures result in a different degree of resistance against chemical agents. *Aspergillus niger*, called black mold, consisting of filament-like strands of cells called hyphae, was treated with silanols and alcohols.

3.2 Experiment

3.2.1 Bacterial Preparations

The bacterial strains employed were the Gram-negative bacteria, *Escherichia coli* (*E.coli*) C3000 (ATCC 15597) and a laboratory strain of *Staphylococcus aureus* (*S.aureus*) received from the Department of Microbiology at the University of Florida and the Gram-positive bacteria, *Pseudomonas aeruginosa* (*P.aeruginosa*) type strain (ATCC 10145) and *Enterococcus faecalis* (*E. faecalis*) type strain (ATCC 19433). The

bacteria suspensions were prepared according to the procedure by Rincon et al (2003). 90 ml of deionized water and Columbia broth nutrient solution were sterilized in an autoclave at a pressure of 15 lb/in^2, and a temperature of 121 ºC, for 30-60 minutes. The bacteria were inoculated into a flask containing 90 ml of autoclaved water and 10 ml of Columbia broth and incubated for 20-24 hours at 37 ºC with constant agitation, 200rpm, under aerobic conditions. The bacterial cells were collected by centrifugation at 500rcf (relative centrifugal force) for 10 minutes at 4 ºC and washed 3 times with sterilized deionized water. A bacterial pellet was resuspended in the sterilized water after final washing. The concentrations of the bacteria suspension prepared were 2-6 $\times 10^8$ cfu/ml (colony forming units/ml).

3.2.2 Antimicrobial Test Procedures and Viability Test

Minimum lethal concentrations (MLC), the so called minimum bactericidal concentration (Collines, 1995), defined as the maximum dilution of the product that kills the test organisms by more than a 7-log reduction after an one hour exposure was used as a measure of the antimicrobial activity of the silanols, the alcohols, and the phenols. Log reduction was measured for materials showing a weak antimicrobial activity. Log reduction stands for a 10-fold or 90% reduction in the number of bacteria. The antimicrobial activity tests were carried out in aqueous condition (Cremieux, 2001) by adding a given concentration of antimicrobial agent to 9 ml of medium, deionized water, and 1ml of bacterial suspension that contains a concentration of 2-6 $\times 10^8$ cfu/ml. Phase separation between oily phase from the antimicrobials and aqueous phase from the medium is expected depending on the hydrophobicity of the antimicrobials. The solution was mechanically mixed for an hour with constant stirring at room temperature to

overcome the phase separation and make bacteria to be exposed to the antimicrobials. One ml sample was collected after 1 hour treatment for a viability test.

The viability test method of the treated bacteria was the agar plate-count method (Collines, 1995; Franson, 1985). The number of bacteria growing on the plate is potentially up to 10^7 cfu/ml if all the treated bacteria survived. Serial dilutions of the sample prior to plating can make the colonies growing on the plate countable. Since one colony arises from a single bacterium, the number of colonies counted on the plate is as same as the number of viable bacteria, referred to as colony forming units (CFU), in the sample. For statistical accuracy, only plates containing between 30 and 300 colonies were counted. Samples were diluted in phosphate buffered saline based on a serial 1/10th dilution. 0.1ml from each of the last two dilution tubes was plated on plate-count agar (Difco) based on the spread plate method (Atlas, 1986; Franson, 1985). After 24 hours of incubating the plates at 37 ºC, the colonies that grew on the medium were counted to estimate the number of viable bacteria. Control runs were conducted under the same conditions except the antimicrobial agents were not included. The log reduction was calculated by subtracting the number of viable bacteria that were treated from the number of bacteria from control experiment. Error bars in the figures were determined by taking a mean of 3-5 tests. Minimum lethal concentration (MLC) for each agent was the lowest concentration of an agent that killed test organisms by more than a 7-log reduction. The serial dilution was not required for obtaining the MLC because a complete killing, in this case a 7-log reduction, of test bacteria was measured.

3.2.3 Time, Bacterial Concentration, and Strain Dependent Test

Experiments were performed to determine how the minimum lethal concentration value varied depending upon exposure time, bacterial concentration, and bacterial strain.

The obtained results can be compared with the minimum lethal concentration data obtained at a given experimental set-up of a 1 hour exposure time and a bacterial concentration of $2\text{-}6\times10^8$ cfu/ml. The antimicrobial and viability test procedures are described in section 3.2.2.

Time dependent experiments were conducted by testing four silanols including n-propyl-, n-butyl-, benzyl-, and phenethyldimethylsilanol against *Escherichia coli* at a fixed bacterial concentration of $2\text{-}6\times10^8$ cfu/ml. The maximum exposure time was limited to 24 hours. The minimum lethal concentrations of the four silanols were obtained at different exposure times. Bacterial concentration of *Escherichia coli* was adjusted from 10^8 to 10^6 and 10^4 cfu/ml to investigate MLC change upon n-butyldimethylsilanol and triethylsilanol test for 1 hour contact time. For the strain dependent test, different strains of *Escherichia coli*, C-3000 and C, and *Staphylococcus aureus*, Smith compact and standard laboratory strain, were examined against n-propyl-, n-butyl-, benzyl-, and phenethyldimethylsilanol. *Escherichia coli* C strain and *Staphylococcus aureus* Smith compact were identified and obtained from the Department of Microbiology at the University of Florida. *Escherichia coli* strains have different history of depositors and isolation. Both *Escherichia coli* strains were found to be coliphages meaning virus infecting *Escherichia coli*. *Escherichia coli* C is somatic (F-) meaning the infection occurs through receptors on lipopolysaccharide, whereas *Escherichia coli* C-3000 is male specific (F+) meaning the infection happens through receptors on pili (Scott et al., 2002). In the case of *Staphylococcus aureus* the Smith compact strain is a capsuleless isogenic strain of Smith diffuse. In contrast the surface of Smith diffuse strain is covered with thick layer of fine fibrous structures (Arizono et al., 1991).

3.2.4 Cosolvent Effects

The antimicrobial activity tests were performed in aqueous solution for 1 hour, consisting of a given concentration of antimicrobial agent, 8.5ml of deionized water, 1ml of bacterial suspension, and 0.5ml of glycol ether, 4.5-4.6 (% g/g) solvent. Propylene glycol ethyl ether, propylene glycol monomethyl ether, and propylene glycol n-propyl ether were evaluated for a study of the cosolvent effect.

3.2.5 Fungicidal Test

Fungicidal activity of silanols was examined against *Aspergillus niger* and compared with analogous alcohols. The minimum fungicidal concentration was determined using the broth dilution method (Swart, 2001). In this method, a given amount of fungicidal agent was added into a flask containing 1ml of fungus suspension and 9ml of liquid medium, Columbia broth solution, that can support the growth of the fungus. The flask was placed in a shaker for incubation at 37 ºC or at room temperature for 24 hours with constant shaking. A sample was collected from the solution and plated on an agar growth medium based on the spread plate method (Franson, 1985). For the control experiment, the serial dilution method was used to measure the concentration of the fungus. The prepared concentration of *Aspergillus niger* was $2\text{-}4\times10^5$ cfu/ml. The lowest concentration of the agent that showed no visible growth on the agar plate after 24 hours of incubation, showed a 4-log reduction and is the minimum fungicidal concentration (MFC).

3.3 Results and Discussion

3.3.1 Antimicrobial Activities of Silanols, Alcohols, and Phenols

The minimum lethal concentrations (MLC) of the silanols, alcohols, and phenols were determined for the Gram-negative bacteria, *Escherichia coli* and *Psedomonas*

aeruginosa and the Gram-positive bacteria, *Staphylococcus aureus* and *Enterococcus faecalis* as summarized in Table 3-1. Figure 3-2, 3-3, and 3-4 shows the obtained MLC for each series of silanols, alcohols and phenols for four bacteria strain respectively. A change in the MLC value was observed as a substituent of the agents varied. It should be pointed out that the lower the minimum lethal concentration, the higher is the antimicrobial activity. A reduction in the MLC value was clearly demonstrated when a substituent of each material was altered from a short alkyl subtituent, such as methyl or ethyl, to a longer alkyl chain like propyl or butyl of silanols, alcohols, and phenols. In addition to the alkyl substituents, vinyl and aromatic substituents including phenyl, benzyl, and phenethyl were also evaluated as well as chlorine substituent for phenols. In the case of aromatic substitutents, phenethyl showed higher antimicrobial activity than that of benzyl followed by phenyl. It can be concluded that a longer alkyl chain of substituent in a series of silanols, alcohols, and phenols resulted in an enhanced antimicrobial activity. We hypothesized that a change of the physicochemical properties of silanols, alcohols, and phenols when a substituent varies, contributes to either an increase or a decrease of antimicrobial activity. The relationship between the antimicrobial activities and the physicochemical properties is presented in Chapter 4.

The susceptibility of the bacteria can be compared based on the relative minimum lethal concentration needed. The MLC for each bacterium exposed to the silanols only slightly varied as shown in Figure 3-2, indicating that the resistance of each bacterium was relatively similar. The degree of the resistance of the bacteria tested against the alcohols and the phenols were also similar to the silanols results as presented in Figure 3-3 and 3-4. *Enterococcus faecalis*, however, was overall the least susceptible bacterium

followed by *Escherichia coli*, *Psedomanoas aeruginosa*, and *Staphylococcus aureus*. The susceptibility of each bacterium may be related to their chemical structure and the components of the cell wall or the membrane. The structure of the cell wall and the cell membrane of the Gram-negative bacteria are different from the Gram-positive bacteria as previously discussed (Atlas, 1984). Bacteria containing more lipid content were reported to be more resistance against the antibiotics such as benzylpenicillin or cloxacillin (Hugo and Stretton, 1966; Norris et al., 1985) because the lipid can protect the bacteria from lipophilic compounds as well as very hydrophilic compounds (Lien et al., 1968). Lipid content could be one of the reasons why the resistances of the tested bacteria varied even though the differences were low.

The silanols showed at least two times more effective antimicrobial activity than the analogous alcohols against the four bacteria based on the MLC values presented in Table 3-1. For *Escherichia coli* test, trimethylsilanol and phenethyldimethylsilanol showed 2.36 % and 0.1% of MLC respectively, whereas the corresponding alcohols, *t*-butanol and phenethyldimethylcarbinol exhibited 13.54% and 0.26% of MLC respectively. It was initially proposed that the physicochemical properties of the silanols, in particular, the higher hydrophobicity and the higher H-bond acidity when compared to the alcohols, contribute to the enhanced bioactivity of the silanols. Hunt (1975) and McKarns et al. (1997) claimed that the degree of potency of the materials was related to their lipophilicity because lipid solubility or membrane permeation of the cell is directly correlated to the magnitude of the hydrophobic interaction between the hydrophobic group of molecule and lipid region in the membrane.

The importance of the balance between the hydrophilic and hydrophobic portions of the compounds has also been suggested for the antibacterial activity in studies related to antimicrobial effect of alcohols, (Kubo et al., 1993a). Kubo proposed that the hydrophobic interaction allows the lipophilic portion of the alcohols to enter the cell membrane and the polar hydroxyl groups are oriented into the aqueous phase by hydrogen bonding. The hydrogen bonding can be formed with ester linkages of fatty-acyl residues and with water molecules (Dombek and Ingram, 1984). The hydrogen bonding forces are directly related to the H-bond acidity (West and Baney, 1959). Alcohols such as ethyldimethylcarbinol having a higher partition coefficient of 1.22 compared to that of trimethylsilanol 1.14 as shown in Table 2-2 demonstrated lower antimicrobial activity. *n*-butyldimethylsilanol with a log P of 2.85 also showed a lower activity than that of 4-ethylphenol, log P of 2.55. The higher antimicrobial activity of trimethylsilanol and 4-ethylphenol may be attributed to their higher H-bond acidity, suggesting that the contribution of the H-bond acidity is significant. 4-hexyl phenol which contains the most carbon atoms out of all the compounds tested in this study showed the lowest minimum lethal concentration, 0.004% against *Staphylococcus aureus*, 0.006% against *Enterococcus faecalis*.

A fall-off of antimicrobial activity was observed as the number of carbon atoms of the substituent of the silanols, the alcohols, and the phenols increased respectively. The antimicrobial activity of 4-hexyl phenol 0.055% of MLC was lower than that of 4-pentyl phenol value of 0.01% against *Escherichia coli*. Benzyldimethylcarbinol having a MLC of 0.58% against *Psedomanoas aeruginosa* also showed a lower activity compared to the value of 0.52% for butyldimethylcarbinol. Phenethyldimethylcarbinol also exhibited a

continuous decrease in the activity after benzyldimethylcarbinol against *Psedomanoas aeruginosa* as shown in Table 3-1. The minimum lethal concentrations for 4-hexyl, 4-pentyl phenol and n-butyl, benzyl, phenethyldimethylsilanol were not able to be determined against *Psedomanoas aeruginosa* as a result of a significant reduction or disappearance of antimicrobial activity of the compounds as the hydrocarbon chain length of the subtitutent increases.

Table 3-1. Minimum lethal concentrations of silanols, alcohols, and phenols against *Escherichia coli*, *Staphylococcus aureus*, *Psudomanos aeruginosa*, and *Enterococcus faecalis*. Each minimum lethal concentration is the averaged value out of 3 data points.

		Minimum lethal concentration (% g/g)			
Materials	R	*E.coli*	*S.aureus*	*P.aeruginosa*	*E.faecalis*
Silanols	Methyl	2.36±0.12	2.48±0.17	2.36±0.01	3.15±0.05
$R(CH_3)_2SiOH$	Vinyl	1.23±0.06	1.04±0.07	1.0±0.03	1.32±0.03
	Ethyl	1.04±0.04	0.80±0.02	0.87±0.04	1.14±0.05
	n-propyl	0.43±0.03	0.36±0.04	0.40±0.03	0.50±0.05
	Phenyl	0.27±0.03	0.26±0.03	0.35±0.05	0.42±0.03
	Benzyl	0.17±0.01	0.16±0.01	×	0.23±0.03
	n-butyl	0.14±0.01	0.14±0.01	×	×
	Phenethyl	0.10±0.02	0.08±0.02	×	0.12±0.02
Alcohols	Methyl	13.54±1.27	10.61±0.19	9.79±0.19	13.33±0.42
$R(CH_3)_2COH$	Vinyl	5.23±0.06	4.37±0.05	3.67±0.08	5.63±0.18
	Ethyl	5.09±0.04	4.17±0.12	3.96±0.04	5.67±0.15
	n-propyl	1.68±0.04	1.69±0.04	1.03±0.03	1.76±0.04
	Phenyl	0.96±0.06	0.78±0.03	0.71±0.03	0.93±0.03
	n-butyl	0.67±0.03	0.65±0.02	0.53±0.02	0.76±0.06
	Benzyl	0.7±0.05	0.59±0.02	0.63±0.03	0.75±0.05
	Phenethyl	0.26±0.01	0.26±0.01	1.33±0.28	0.32±0.02
Phenols	[1]Hydrido	0.70±0.01	0.61±0.02	0.62±0.08	0.98±0.03
RC_6H_5OH	4-methyl	0.41±0.01	0.35±0.02	0.35±0.05	0.45±0.05
	3-chloro	0.13±0.02	0.11±0.01	0.11±0.01	0.14±0.01
	4-ethyl	0.13±0.02	0.14±0.02	0.11±0.01	0.17±0.03
	4-propyl	0.053±0.006	0.045±0.005	0.052±0.008	0.055±0.005
	2-phenyl	0.12±0.03	0.085±0.015	0.13±0.03	0.13±0.03
	4-butyl	0.013±0.002	0.015±0.005	0.06±0.01	0.017±0.003
	4-pentyl	0.010±0.002	0.008±0.002	×	0.012±0.002
	4-hexyl	0.055±0.005	0.004±0.001	×	0.006±0.001

[1] Hydrido : unsubstituted phenol. × indicates no minimum lethal concentration obtained.

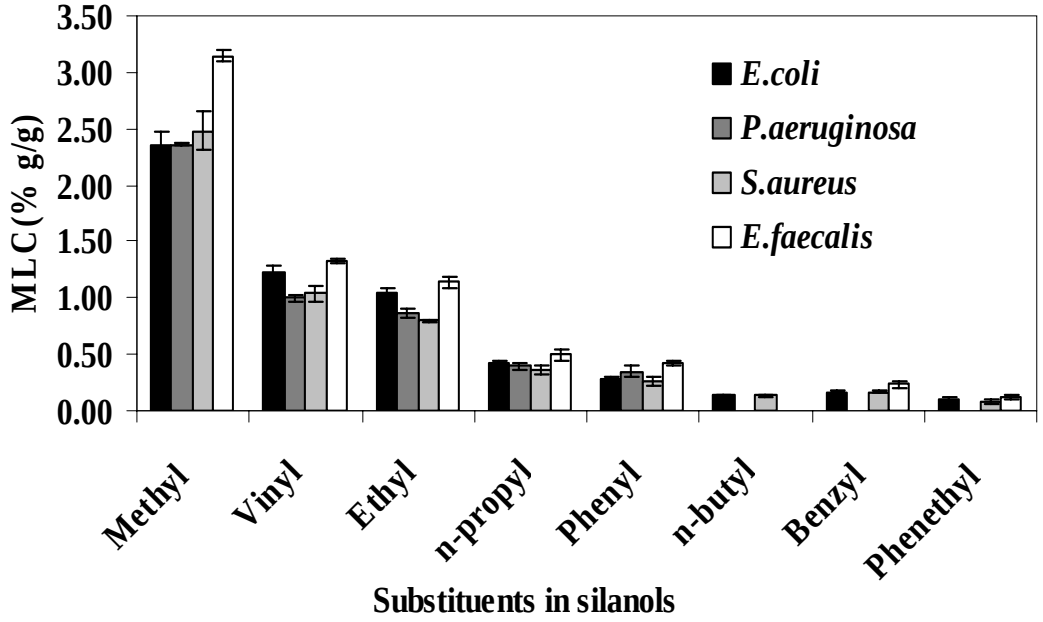


Figure 3-2. Minimum lethal concentration of the silanols ($R(CH_3)_2SiOH$) against Gram-negative bacteria, *Escherichia coli* and *Psedomonas aeruginosa* and Gram-positive bacteria, *Staphylococcus aureus* and *Enterococcus faecalis*

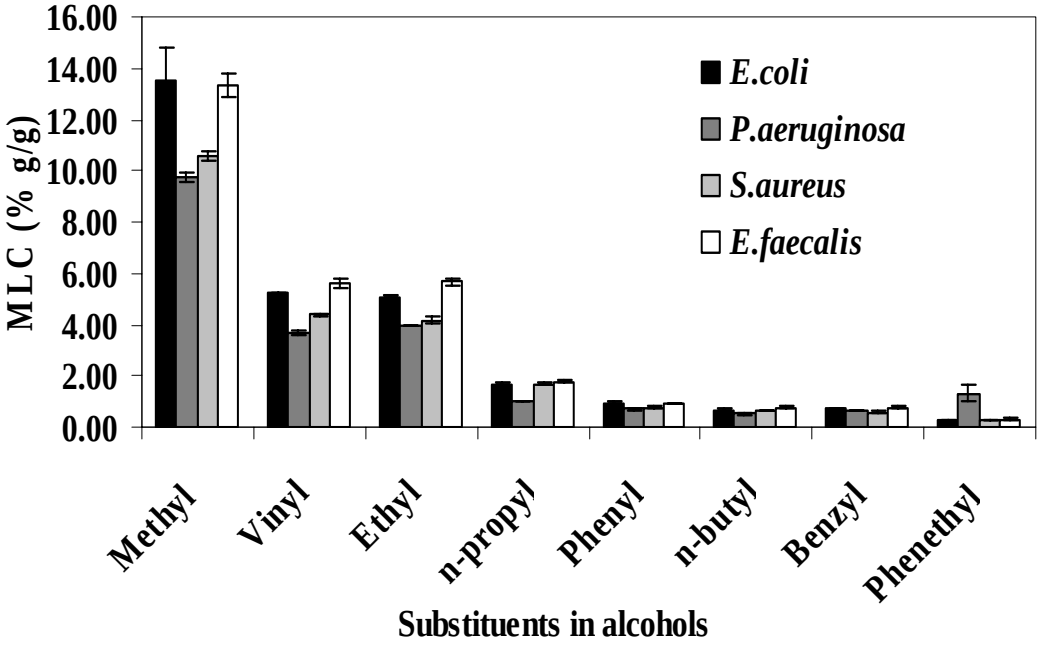


Figure 3-3. Minimum lethal concentration of alcohols ($R(CH_3)_2COH$) against Gram-negative bacteria, *Escherichia coli* and *Psedomonas aeruginosa* and Gram-positive bacteria, *Staphylococcus aureus* and *Enterococcus faecalis*

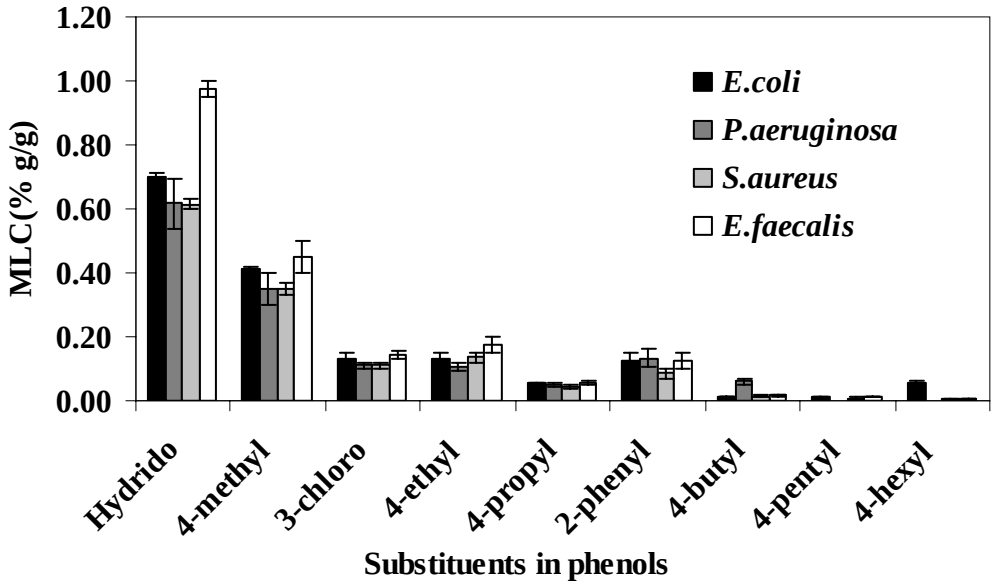


Figure 3-4. Minimum lethal concentration of substituted phenols against Gram-negative bacteria, *Escherichia coli* and *Psedomonas aeruginosa* and Gram-positive bacteria, *Staphylococcus aureus* and *Enterococcus faecalis*

3.3.2 Antimicrobial Activities of the Impurity of Silanols – The Disiloxanes

Silanols were prepared through the hydrolysis of chlorosilanes. The purities of the silanols measured by Si-29 and H-1 NMR (Nuclear Magnetic Resonance spectroscopy) method are approximately 95 ± 3 % with the impurity consisting of disiloxanes arising from condensation of silanols as shown in Table 2-1. Antimicrobial activities of disiloxanes such as hexamethyldisiloxane impurity in trimethylsilanol, hexaethyldisiloxane impurity in triethylsilanol, divinyltetramethyldisiloxane in vinyldimethylsilanol, and diphenyltetramethyldisiloxane in phenyldimethylsilanol were evaluated to determine their contribution to the antimicrobial activities of the silanols. The experiments were performed using 10 (%g/g) of the disiloxanes. However, for example, the actual concentration of hexamethyldisiloxane in the case of taking 2.4% of trimethylsilanol is no more than 0.12%. Therefore, 10% of hexamethyldisiloxane is 100 times more than that of the actual concentration of the impurity for the antimicrobial

activity test of trimethylsilanol. The log reduction was observed against four different bacteria, *Escherichia coli*, *Psedomonas aeruginosa*, *Staphylococcus aureus*, and *Enterococcus faecalis*. The disiloxanes showed a low antimicrobial activity against the four bacteria at a concentration of 10%, less than a 1-log reduction as shown in Figure 3-5. The log reduction by the impurities can be 100 times less than for the actual experiment, illustrating that the silanols were responsible for the 7-log reduction of the viable bacteria.

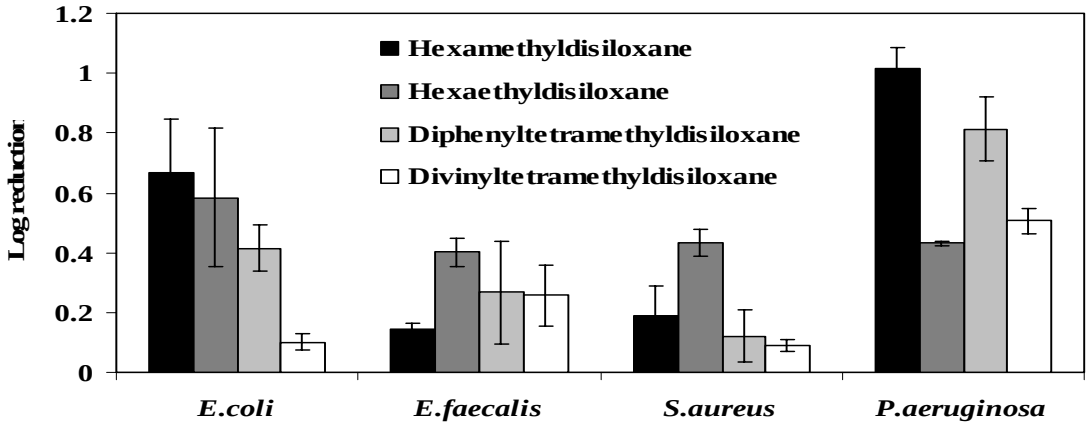


Figure 3-5. Log reduction of disiloxanes at 10%(g/g) against *Escherichia coli*, *Psedomonas aeruginosa*, *Staphylococcus aureus*, and *Enterococcus faecalis*.

3.3.3 A Variation of the Minimum Lethal Concentration Depending on Contact Time, Bacterial Concentration, and Bacterial Strain

Time dependent test. All the minimum lethal concentration data were measured at a given experimental set-up of 1 hour mixing time with a concentration of bacteria 2-6×10^8 cfu/ml. The variation of the minimum lethal concentration with exposure time was investigated in order to understand the relationship between exposure time and the MLC required for inactivation of the bacteria. The MLCs of the silanols required to kill *Escherichia coli* C-3000 with a 7-log reduction, were reduced by approximately 12-20% for 2 hours and by 25-35% for 24 hours exposure time as presented in Figure 3-6.

Bakker-Woudenberg et al. (2005) reported that the concentration needed to achieve bactericidal activity of moxifloxacin within 3 days was 16 times higher compared to the concentration needed at 21 days of exposure. A novel semisynthetic cyclic glycopeptide antibiotic also demonstrated time-dependent killing by varying the concentration of the antibiotic (Petersen et al., 2004). These studies showed that an increase in the concentration of the antibiotic led to a reduction of exposure time for equal bactericidal effects. The MLC decreased relatively linearly as exposure time increases within 3-5 hours, but the rate of decrease of the MLC becomes unnoticeable after 5 hours exposure until the exposure time reaches 24 hours or more, suggesting that an optimum concentration for a given set-up may exist for each material regardless of exposure time.

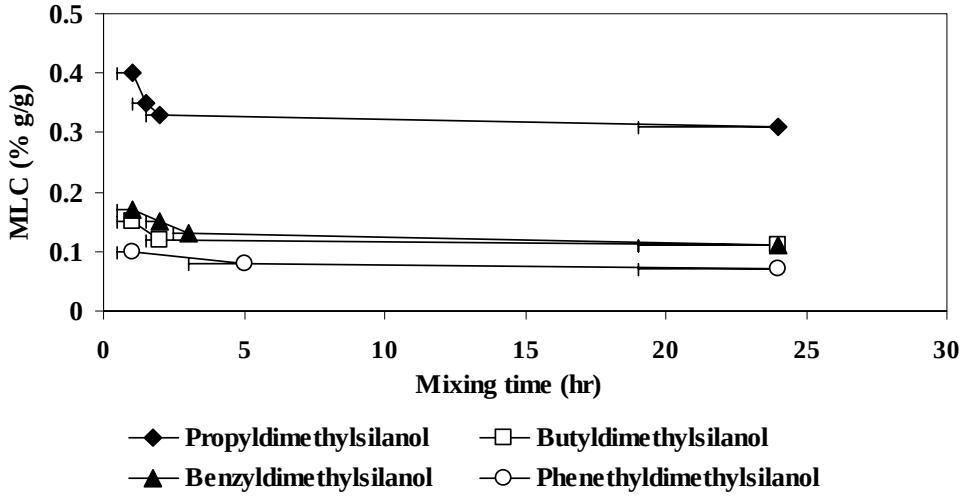


Figure 3-6. The minimum lethal concentrations of silanols dependent on mixing time against *Escherichia coli*.

Bacterial concentration dependent test. A change in the minimum lethal concentration was demonstrated as a function of the bacterial concentration. The MLC represents a 3-log reduction, 5-log reduction, and 7-log reduction in this test because the prepared bacterial concentrations were 10^4 cfu/ml, 10^6 cfu/ml, and 10^8 cfu/ml respectively. *Escherichia coli* C-3000 prepared at different concentrations was tested

with *n*-butyldimethylsilanol and triethylsilanol. As expected, the MLCs needed decreased by approximately 23-29% for 10^4 cfu/ml and 14-17% for 10^6 cfu/ml compared to that of 10^8 cfu/ml of *Escherichia coli* as seen in Figure 3-7.

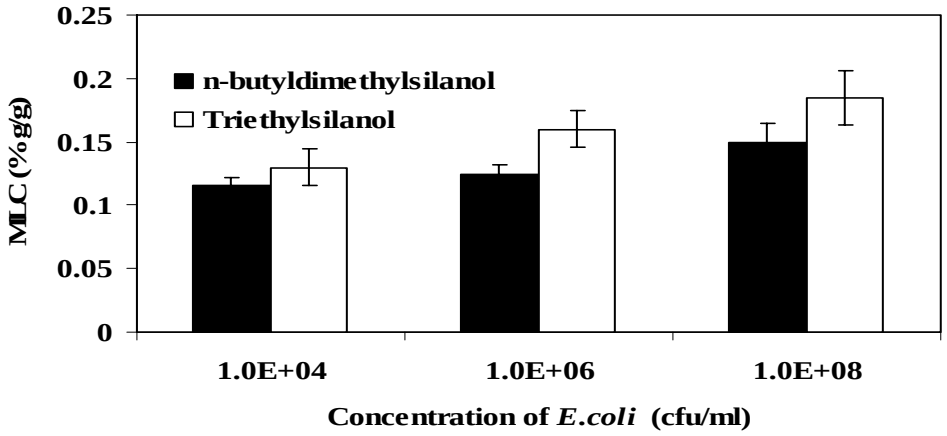


Figure 3-7. The minimum lethal concentrations of the silanols at different bacterial concentration against *Escherichia coli*.

Strain dependent test. A strain is a subset of a bacteria species differing from other bacteria of the same species. Strains within a species may differ slightly from one another in many ways due to their origin or growing conditions such as temperature, pH, and nutrient (Atlas, 1984). The resistance of bacteria strains against chemical agents often varies from strain to strain (Bozdogan et al., 2004; Chen et al., 2004). For example, *Escherichia coli* strains 9061, 9062, and 9065 isolated from baby pigs were treated by bovine lactoferrin hydrolysate combined with other agents such as penicilline G and cephalothin. Only strain 9061 was susceptible to cephalothin whereas other strains were susceptible to other antimicrobial agents (Chen et al., 2004). In our study, differences in susceptibility among different strains were small. *Staphylococcus aureus* Smith compact has slightly higher resistance than that of the laboratory strain as shown in Figure 3-8. In the case of *Escherichia coli* strains, C-3000 strain showed a little higher resistance than that of the C strain as seen in Figure 3-9.

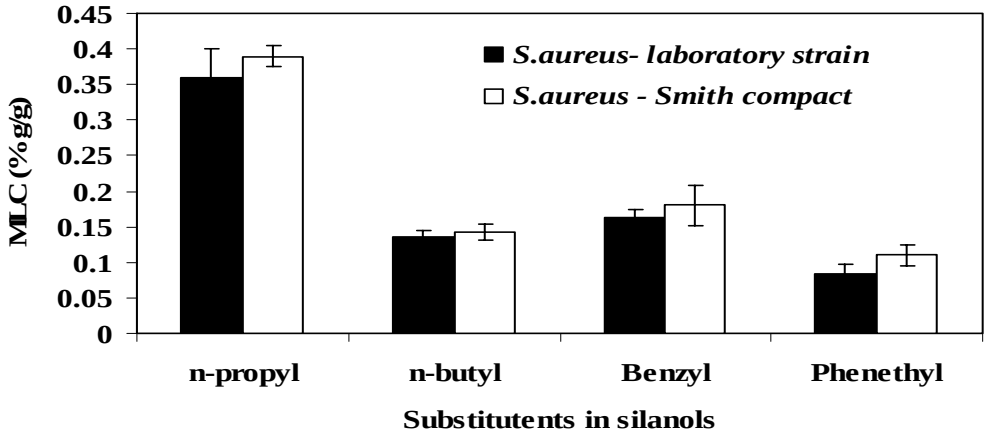


Figure 3-8. Strain dependent test results – MLC of *Staphylococcus aureus* strains, Smith compact and standard laboratory strain (Department of Microbiology at the University of Florida).

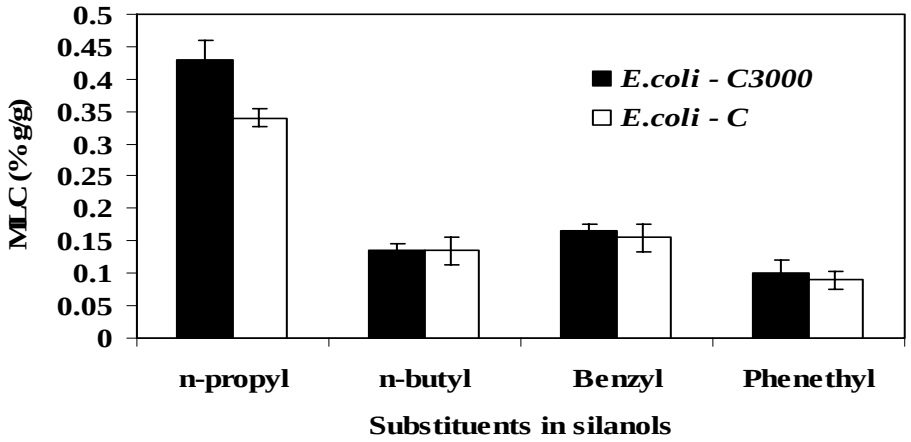


Figure 3-9. Strain dependent test results – MLC of *Escherichia coli* strains, C-3000 and C strain

3.3.4 Cosolvent Effect

The use of solvents other than water as cosolvent to an aqueous phase can affect antimicrobial activity by the inherent toxicity of the cosolvent or through the effect of cosolvent on the solubility of antimicrobial agents. Experiments were carried out to improve the antimicrobial activity of silanols by increasing their solubility with a compatablizing cosolvent. Three different propylene glycol alkyl ethers were utilized

because propylene glycol ether has an excellent balance between hydrophilic and hydrophobic properties.

The concentration of the cosolvent was limited to a range of 4.5-4.6 (% g/g), a concentration at and below which it killed bacteria with less than a 1-log reduction. The reduction % of *Escherichia coli* at the concentration of 4.5-4.6 % of the cosolvent varied depending on the partition coefficient log P of the propylene glycol alkyl ether as shown in Table 3-3. Propylene glycol *n*-propyl ether showed 49±7.9% of bacterium reduction, 17.8±5.0% for propylene glycol ethyl ether, and 3.1±2.2% for propylene glycol methyl ether. In comparison, the minimum lethal concentrations required for silanols mixed with the cosolvent were reduced by 57-49% for propylene glycol *n*-propyl ether, 26-11% for propylene glycol ethyl ether, and 15-5% for propylene glycol methyl ether, summarized in Table 3-2. The extent of reduction of the MLC was consistent with the bacterial reduction by cosolvent at a given concentration. Hence, an enhancement of antimicrobial activity of the cosolvent system appears to be closely related to an inherent toxicity of each cosolvent. The antimicrobial activity of the cosolvents was linearly related to their partition coefficient. Minimum lethal concentrations of the cosolvents were estimated by the correlation equations presented in Chapter 4 using their structural parameters displayed in Table 3-3. Predicted MLCs for each cosolvent were much higher than the dosages, 4.5-4.6 (%g/g), we used for cosolvent experiment. Table 3-3 showed the calculated bacterial reduction based on difference between the actual dosage and the predicted MLCs. The observed values of bacterial reduction % of the cosolvent were similar to those of the calculated values.

The cosolvents affected the solubility of the silanols and improved the antimicrobial activity of long alkyl chain silanols. The minimum lethal concentration of *n*-pentyldimethylsilanol and *n*-hexyldimethylsilanol could not be detected at the range from 0.1 to 10 (% g/g) of concentration due to the lack of their solubility in water as well as in the bacteria membrane. However, when using 4.5 (% g/g) of propylene glycol *n*-propyl ether *n*-pentyldimethylsilanol and *n*-hexyldimethylsilanol showed an increase of antimicrobial activity with a value of 0.03 (% g/g) MLC and 0.05 (% g/g) MLC respectively against *Escherichia coli*. Propylene glycol was used as a permeation enhancer for improving transdermal drug delivery (Megrab et al., 1995b). The authors claimed that the uptake of the lipophilic drug oestradiol was enhanced in the presence of propylene glycol as a cosolvent, due to an increased solubility of the oestradiol in the membrane (Megrab et al., 1995b). A similar experiment performed with ethanol as a cosolvent showed an increase in the drug uptake as a result of increased drug solubility in the stratum corneum (Megrab et al., 1995a). Megrab et al. (1995a) and Williams et al. (2004) also claimed that permeation of solvent like ethanol or propylene glycol into the cell could alter the solubility properties of the tissue causing fluidization of the lipids, as a consequence drug partitioning into the membrane can be improved. We theorize that the enhanced activity was a result of improved solubility of the long alkyl chain silanols in water and the bacteria membrane.

In Table 3-2, R % is from 100×(MLC of without cosolvent - MLC of with cosolvent)/MLC of without solvent. The equations used for calculating the MLC values in Table 3-3 are:

$$\text{Log}(1/\text{MLC1})=0.739\text{logP}-1.294 \qquad (3\text{-}1)$$

$$\text{Log}(1/\text{MLC2})=0.63\log P+0.0037\,\Delta\nu-1.84 \qquad (3\text{-}2)$$

Bacterial reduction % calculated from 100×(Dosage/Predicted MLC), reduction %1 values were based on MLC1 and reduction %2 values were based on MLC2.

Table 3-2. Minimum lethal concentrations of silanols and silanols mixed with propylene glycol alkyl ethers (4.5-4.6 (% g/g)). R% between silanols and silanols with the cosolvent.

	Propylene glycol n-alkyl ether						
$R(CH_3)_2SiOH$	Propylene glycol n-propyl ether		Propylene glycol ethyl ether		Propylene glycol methyl ether		without solvent
R	MLC	R %	MLC	R %	MLC	R %	MLC
Ethyl	0.53±0.04	49	0.78±0.1	26	0.89±0.09	15	1.04±0.04
n-propyl	0.22±0.02	50	0.34±0.03	21	0.38±0.03	12	0.43±0.03
n-butyl	0.063±0.004	55	0.125±0.007	11	0.133±0.004	5	0.14±0.01
phenethyl	0.043±0.004	57	0.075±0.007	25	0.093±0.004	8	0.1±0.02

Table 3-3. Comparison of the data between the bacterial reduction % observed and calculated based on prediction models provided in Chapter 4 and Table 4-3.

			Structural properties		Predicted MLC		Bacterial reduction % calculated	
Solvent	Dosage (% g/g)	Bacterial reduction % - observed	log P	Δν	MLC1	MLC2	Reduction % 1	Reduction % 2
Propylene glycol n-propyl ether	4.5	49±7.9	0.49	107	8.54	13.65	52.7	33
Propylene glycol ethyl ether	4.6	17.8±5.0	0.002	110	19.6	27.0	23.5	17
Propylene glycol methyl ether	4.6	3.1±2.2	-0.49	111	45.3	54.7	10.2	8.4

It has been mentioned that the phase separation of the antimicrobial from the medium occurs due to the hydrophobicity of the antimicrobials. A problem with the phase separation might be an availability of the antimicrobial to bacteria may not be as same as the homogenous system. A cosolvent, propylene glycol n-propyl ether, which has a good balance between the hydrophilic and the hydrophobic properties, was used to improve their solubility in aqueous phase. The minimum lethal concentrations of eight of silanols and analogous alcohols mixed with 4.5 (% g/g) of propylene glycol n-propyl

ether were measured and the comparison results are presented in Figure 3-10 and 3-11. A reduction of the MLC of the antimicrobials with the cosolvent was approximately 52% for the silanols and 54% for the alcohols when compared to the antimicrobials without the cosolvent. The magnitude of the reduction of the MLCs is similar among the tested silanols and alcohols which have a different hydrophobic property, indicating that the potential problem with the phase separation of the antimicrobials is not significant because the availability of the antimicrobials in the solubility improved system was similar to the inhomogeneous system.

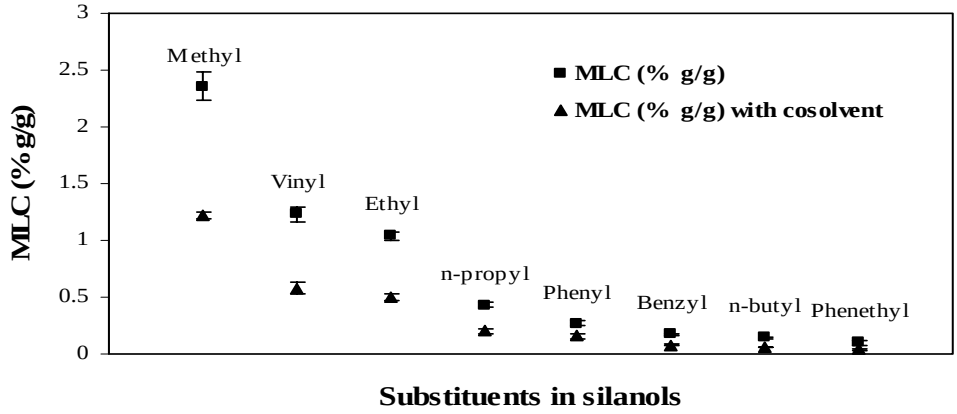


Figure 3-10. Antimicrobial activity of silanols against *Escherichia coli* with a cosolvent, propylene glycol n-propyl ether, at concentration of 4.5 (% g/g), compared to silanols without a cosolvent.

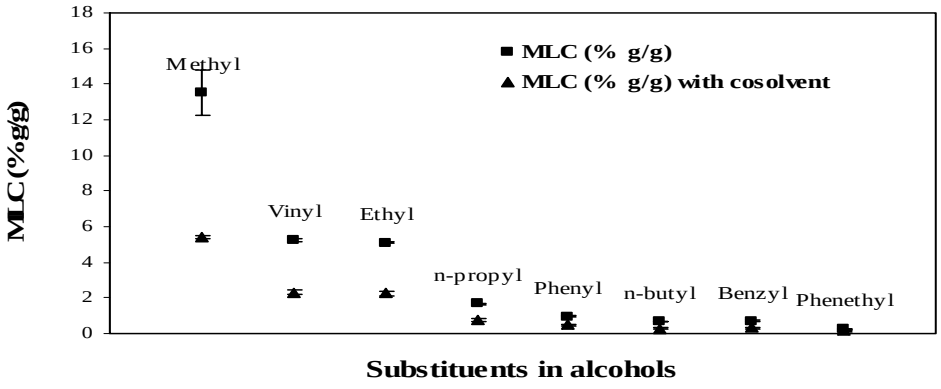


Figure 3-11. Antimicrobial activity of alcohols against *Escherichia coli* with a cosolvent, propylene glycol n-propyl ether, at concentration of 4.5 (% g/g) compared to alcohols without a cosolvent.

3.3.5 Fungicidal Activities of Silanols and Alcohols

The fungicidal activities of silanols against *Aspergillus niger* (black mold) were compared with analogous alcohols as summarized in Table 3-4. Tested silanols required a lower concentration than those for analogous alcohols as shown in Table 3-4, similar to the results observed in the antibacterial activity test with silanols compared to alcohols. However the susceptibility of *Aspergillus niger* was relatively higher than that of the bacteria, *Escherichia coli* C-3000 or *Staphylcoccus aureus* laboratory strain as shown in Figure 3-12. It should be mentioned that the fungicidal acidity test was for a 24 hours mixing period instead of 1 hour mixing period. The fungi's higher resistance was reported previously by Karbit et al.(1985) on phenol experiment. 0.5% phenol solution was tested for treatment of fungi and bacteria at a pH of 6.1 and at room temperature. D-values in hour which is the amount of time that it takes at room temperature to kill 90% of the organisms, were 32.4 hour for *Aspergillus niger*, 1.72 hour for *Escherichia coli*, and 1.90 hour for *Staphylococcus aureus*.

In this study, the minimum fungicidal concentrations were measured at different incubation temperatures, 37 ºC and room temperature, to determine the temperature dependence. Table 3-4 compares the reduction of the fungicial activity at room temperature with at 37 ºC. The fungicidal effect of *n*-butyldimethylsilanol at room temperature was not detected at a concentration of 5 (%g/g) even though it showed 0.55 (% g/g) of MFC at 37 ºC indicating a significant reduction of fungicidal activity of *n*-butyldimethylsilanol at room temperature. The temperature dependence was examined by several scientists (Karabit et al., 1985; Leitch and Stewart, 2002a, b). Karabit et al. (1985) demonstrated a linear relationship between the logarithmic of the death rate constant and the reciprocal of the absolute temperature, which suggests that the activity decreases as

temperature decreases. The susceptibility of *Escherichia coli* O157 against lactate was also tested at different temperatures, 37 ºC, 20 ºC, and 5 ºC (Leitch and Stewart, 2002a, b). The authors reported that the activity of lactate was reduced at lower temperatures due to the reduction in membrane potential and intracellular lactate anion concentration. According to the lactate experiment, it is possible that the uptake of silanols at room temperature was less efficient than that of at 37 ºC so that a higher concentration of silanols was needed to exhibit equal-toxicity.

Table 3-4. Fungicidal activity – Minimum fungicidal concentrations of silanols and alcohols at 37ºC and room temperature.

	MFC(%g/g) - $R(CH_3)_2SiOH$		MFC(%g/g)- $R(CH_3)_2COH$
R	37ºC	Room temperature	37ºC
n-propyl	0.7±0.07	0.9±0.1	2.1±0.14
n-butyl	0.55±0.07		1.1±0.17
Benzyl	0.19±0.02	0.28±0.04	0.7±0.07
Phenethyl	0.14±0.02	0.35±0.07	0.28±0.04

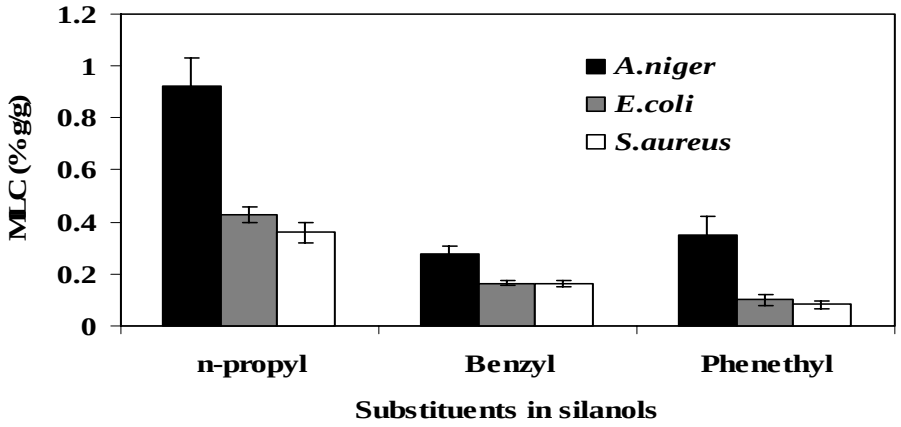


Figure 3-12.Comparison between fungicidal activity and bactericidal activity of silanols at room temperature.

3.4 Conclusions

The minimum lethal concentrations (MLC) of the silanols, a new antimicrobial agent, the alcohols, and the phenols were determined against the Gram-negative bacteria,

Escherichia coli and *Psedomonas aeruginosa* and the Gram-positive bacteria, *Staphylococcus aureus* and *Enterococcus faecalis* at the given conditions. The silanols showed a higher antimicrobial and fungicidal activity than those of their analogous alcohols. It was found that the antimicrobial activity was affected by a substituent of the silanols, the alcohols, and the phenols. The relationship between the antimicrobial activity and the structural properties of the antimicrobials containing a different substituent is reported in Chapter 4. The resistance of each bacterium compared was quite similar against the tested agents. The effects of exposure time, bacterial concentration, and strain of bacteria on the change of minimum lethal concentration were presented. The longer the exposure time, the lower was the MLC required. A reduction of MLC was observed as a bacterial concentration decreased. Different strains of *Escherichia coli* and *Staphylococcus aureus* showed a similar resistance against several silanols. Propylene glycol alkyl ethers used as cosolvent improved the antimicrobial activity of the silanols most likely due to their inherent toxicity based on their physical property and possibly to enhancement of solubility of the silanols.

CHAPTER 4
STRUCTURE-ANTIMICROBIAL ACTIVITY RELATIONSHIP FOR SILANOLS, IN COMPARISON TO ALCOHOLS AND PHENOLS

4.1 Introduction

Understanding the mechanisms of the antimicrobial actions is critical for the development of antimicrobial agents with improved antimicrobial effects. A quantitative study of the relationship between the chemical structure and the biological activity has received considerable attention in the fields of pharmacology and drug development because it allows one to predict chemical toxicity or bioactivity without spending inordinate time and effort (Hansch et al., 1995; Lill et al., 2005; Yen et al., 2005). A method for quantitative biological activity and chemical structure relationship was proposed by Hansch and Fujita in 1964 based on the linear free energy-related approach, called Hansch analysis (Hansch and Fujita, 1964; Hansch et al., 1995). Structural parameters used in the Hansch analysis were linear free energy-related parameters derived from rate or equilibrium constants. A general model, Equation 4-1, was formulated by simplifying the rate-limiting conditions for many biological responses to chemicals. BR is a biological response, C is the molar concentration of applied compound producing a standard response in a fixed time interval, and k_1 is a rate or equilibrium constant for a rate-limiting reaction. The exponential term represents the probability that a molecule reaches a reaction site in time depending on log P. The rate of biological response can be replaced by a constant because the biological response is measured in a fixed time interval. Structural effects are represented by the structural

parameters, k_1, a rate or equilibrium constant, is treated by the so-called linear free energy-related approach as shown Equation 4-2. Lastly, substitution of Equation 4-2 into Equation 4-1 can yield a variety of types of equations such as Equation 4-3, 4-4, 4-5, and 4-6 depending on the types of bioactivity and key structural parameters (Hansch and Fujita, 1964; Hansch et al., 1995).

$$\text{Rate of biological response} = \frac{d(BR)}{dt} = ae^{-\frac{(\log P - \log P_0)^2}{b}}(C)(k_1) \quad (4\text{-}1)$$

$$\log k_1 = \text{a (steric)} + \text{b(electronic)} + \text{c(hydrophobic)+const.} \quad (4\text{-}2)$$

$$\log \tfrac{1}{C} = a \log P + b \quad (4\text{-}3)$$

$$\log \tfrac{1}{C} = a \log P^2 + b \log P + c \quad (4\text{-}4)$$

$$\log \tfrac{1}{C} = a\sigma + c \quad (4\text{-}5)$$

$$\log \tfrac{1}{C} = a \log P + b\sigma + c \quad (4\text{-}6)$$

The Hammett constant σ is defined as $\sigma = \log (K_X/K_H)$ where K_H is the ionization constant of benzoic acid and K_X that of a derivative. σ is a constant that depends on the electronic properties of the substituent X relative to H.

In this study, we focused on determining how the antimicrobial activity changes with variation in the partition coefficient and the H-bond acidity. The expected model is comparable to Equation 4-6. In comparison, the parabolic Equation 4-4 is applied to the case when the tested molecules are too hydrophilic or too lipophilic, so that reduced bioactivities are observed. The fall-off in bioactivity is caused because those molecules can't cross the membranes lipophilic or hydrophilic barriers (Silverman, 2004).

The structural dependence study with antimicrobial activity has been mainly focused on the effects of the lipophilicity through testing a homologous series of samples,

i.e. aliphatic alcohols, alkylated phenol derivatives and quaternary ammonium compounds (Daoud et al., 1983; Klarmann, 1933; Suter, 1941; Tanner, 1943). Tanner et al. (1943) tested the antibacterial activity of aliphatic alcohols containing from 1 to 11 carbon atoms against nine different strains of bacteria. The authors reported that the antibacterial activity increased as the alkyl chain length increased from methyl to pentyl, then, began to decrease as the chain length increase to n-hexyl, heptyl and octyl as a result of a decrease in water solubility. The bactericidal actions of alkyl substituted phenol (Suter, 1941) and the normal alkyl derivatives of *p*-chlorophenols series (Klarmann, 1932) were also reported. Both authors showed that an increase in the alkyl chain length led to an increase in the antimicrobial activity, but a reduction in the activity still occurred as the alkyl chain continue to increase. It was also reported that the cut-off points varied with tested microorganisms. Hansch and Lien (1968) summarized the structure-activity relationship of the antimicrobial agents by means of equations based on a method proposed by Hansch and Fujita in 1964. A multiple regression analysis method was used for developing the equations (Kubinyi, 1993). Hansch and his associates reported that the correlations varied with tested bacteria and types of antimicrobial agents. These authors suggested that the lipophilicity of the compound was the primary factor for the antimicrobial activities of the compounds with a relatively minor contribution from the electronic properties (Lien et al., 1968). Daoud et al. (1983) demonstrated a parabolic relationship between log P and log(1/MIC) of a homologous series of alkyldimethylbenzyl ammonium chlorides, quaternary ammonium salt compounds. MIC stands for minimum inhibition concentration meaning the lowest concentration required for inhibition of bacterial growth.

The correlation models obtained from the previous studies were limited to a series of homologous chemicals such as substituted phenols or benzyl alcohols separately instead of combining different series of chemical compounds. In comparison to the previous works we treated the silanols, the alcohols, and the phenols shown in Figure 2-1 as a single class of antimicrobial agents. The chemical structure of the silanols is composed of a hydrophilic portion formed by hydroxyl group and a hydrophobic region formed by hydrocarbon chains, similar to the alcohols and the phenols as shown in Figure 2-1. We predicted that the mode of antimicrobial actions of the silanols might resemble those of the alcohols and the phenols because of the similarity in the chemical structures of the antimicrobials. In this chapter, the goal is to establish a relationship between the antimicrobial activity and the structural properties of those antimicrobials as a single class of agents. Ultimately, the models can be used for predicting the antimicrobial activity of similar chemicals. A statistical analysis method was utilized to justify the outcomes. External compounds, which were not used for the models, were also evaluated to validate the empirical models.

4.2 Materials and Method

4.2.1 Materials

Twenty-five materials were evaluated with four bacteria, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis* prepared as described in Chapter 3 for the quantitative structure-activity relation models. Silanols, $R(CH_3)_2SiOH$, and alcohols, $R(CH_3)_2COH$, with eight different substituents and nine substituted phenols, $R(C_6H_4)OH$, were evaluated. The substituents, R, prepared were methyl, ethyl, *n*-propyl, *n*-butyl, phenyl, vinyl, benzyl, and phenethyl. The substituents for phenols were 4-methyl, 4-ethyl, 4-propyl, 4-butyl, 4-pentyl, 4-hexyl, 3-chloro, 2-

phenyl. The eleven external compounds for validation of the empirical models were disiloxanediol received from Clariant, triethylsilanol obtained from Gelest Inc., 1,2-octanediol, *n*-hexanol, *n*-heptanol, *n*-octanol, pentyldimethylcarbinol, thymol, and 4-chloro-3-methyl phenol purchased from Acros organics with purities greater than 97%, and 3,3,3-trifluoropropyldimethylsilanol and cyclohexyldimethylsilanol prepared by hydrolysis of chlorine derivative silane. The purity of the prepared 3,3,3-trifluoropropyldimethylsilanol and cyclohexyldimethylsilanol was measured by H-1 and Si-29 NMR, showed no less than 97%.

4.2.2 Parameters and Method for QSAR Model

The minimum lethal concentrations (MLC) displayed in Table 3-1 defined as the concentration required for a 7-log reduction in viable bacteria after 1hour exposure period, were used to measure the antimicrobial activity. The octanol-water partition coefficients (log P) and the H-bond acidities ($\Delta\nu$) reported in Table 2-2 and 2-3, measured as the shift in the infrared frequency of the OH stretching band between free OH and hydrogen bonded OH to diethyl ether oxygen by infrared spectroscopy were utilized as dispersive and polar structural parameters respectively. The MLC values were converted to the logarithms of (1/MLC).Three of log (1/MLC) values for each compound against each bacterium were used for the correlation models and presented in Table 4-2. Correlation between the log (1/MLC) and the structural parameters were established by the multiple regression analysis method. Quick MVR was a small utility program used to calculate a multivariable regression on the obtained data. One of the reasons for using the logarithms of (1/MLC) is to obtain larger values for more active compounds. In this study, the higher the value of log (1/MLC) the stronger is antimicrobial. Another reason is that a condition for the application of regression analysis is a normal distribution of the

experimental error in the dependent variable. In other words, the biological activity values should be reasonably distributed over the whole range, without clustering of data. For biological data this condition holds true for the logarithmic scale (Kubinyi, 1993). As described previously in this chapter, the linear free energy-related approach which was utilizing the logarithmic scale has been used for this quantitative structure-activity study.

4.3 Results and Discussion

4.3.1 Correlation between Antimicrobial Activity and Structural Parameters

It was proposed that a higher hydrophobicity and a higher H-bond acidity of the silanols compared to the analogous alcohols contribute to the enhanced antimicrobial activity of the silanols. Since the substituted phenols are also composed of s hydroxyl group and the hydrophobic organic substituents resemble those of the silanols and the alcohols, the three types of chemical agents were considered as a single class of antimicrobial agents. In this study, a quantitative structure-activity relationship using the silanols, the alcohols, and the phenols was demonstrated. The correlation equations between their antimicrobial activities and their structural properties, log P and H-bond acidity ($\Delta\nu$) presented in Table 4-1 and 4-2, were created by a multiple regression analysis and summarized in Table 4-3. A linear free-energy relationship between the antimicrobial activity, the partition coefficient, and the H-bond acidity was illustrated.

The minimum lethal concentration (MLC) values were converted to the logarithms of reciprocal MLC (log (1/MLC) to demonstrate a linear free-energy relationship with the structural parameters, log P and $\Delta\nu$. Hansch et al. (1995) and Silverman (2004) proposed that a linear free-energy relationship exist between the lipophilicity of chemical compounds using the partition coefficient value and their biological activities. For one structural parameter model, the log (1/MLC) plotted as a function of the partition

coefficient (log P) produced empirical equations as presented in Table 4-3 along with the statistically significant values of correction coefficient r, standard deviation s, and 95% significant level F value. In this study, the structural parameters considered for the antimicrobial activity were not only the partition coefficient but also the H-bond acidity. The partition coefficient is related to the lipophilicity of chemical compounds while the H-bond acidity is dependent on the polar properties of the compounds such as pKa or the Hammitt constant σ and a measure of the hydrogen bond strength. The correlation models with both the H-bond acidity and the partition coefficient are presented in Table 4-3. An increase in the correction coefficient and the F values was clearly observed for the two parameter model when compared to the one parameter model. The significance and validity of the regression equations was evaluated by assessing the correlation coefficient r, the standard deviation s, the F values, and the partial F-test (Kubinyi, 1993; Livingstone, 1995). The correlation models have significant value of the correlation coefficients, support the hypothesis that the silanols, the alcohols, and the phenols are part of the same family, and follow the same kill mechanisms of bacteria such as membrane damage. Since the correlation equations corresponded to each bacterium were similar and follow the same trend the overall equations for the four bacteria with the silanols, the alcohols, and the phenols were obtained, log(1/MLC)= 0.786log P-1.379, n=282, r=0.92, s=0.3, and F=1543, log (1/MLC) = 0.679 log P + 0.0036 $\Delta\nu$ -1.909, n = 282, r = 0.96, s = 0.22, F = 1639, and partial F-test = 268. The overall equations also support the hypothesis that a mechanism of bio-response of the four bacteria with the antimicrobials may be follows the same antimicrobial action.

Table 4-1. Structural parameters, log P and Δν, and antimicrobial activities, log(1/MLC), of silanols, alcohols, and phenols against four bacteria. Each log(1/MLC) is the averaged value of three data point presented in Table 4-2.

		Structural parameters		log(1/MLC) Observed			
Materials	R	log P	Δν	*E.coli*	*S.aureus*	*P.aeruginosa*	*E.faecalis*
Silanols	Methyl	1.14	238	-0.374	-0.395	-0.372	-0.498
$R(CH_3)_2SiOH$	Vinyl	1.5	260	-0.090	-0.017	0.000	-0.122
	Ethyl	1.63	237	-0.017	0.099	0.063	-0.057
	n-propyl	2.12	237	0.370	0.444	0.402	0.301
	Phenyl	2.36	267	0.563	0.579	0.456	0.373
	Benzyl	2.85	257	0.783	0.787	×	0.632
	n-butyl	2.62	236	0.870	0.870	×	×
	Phenethyl	3.34	252	1.000	1.079	×	0.933
Alcohols	Methyl	0.73	126	-1.132	-1.026	-0.991	-1.125
$R(CH_3)_2COH$	Vinyl	1.08	130	-0.719	-0.640	-0.564	-0.750
	Ethyl	1.22	125	-0.706	-0.620	-0.597	-0.754
	n-propyl	1.71	125	-0.225	-0.227	-0.011	-0.245
	Phenyl	1.95	137	0.018	0.108	0.148	0.032
	n-butyl	2.2	126	0.172	0.186	0.279	0.117
	Benzyl	2.44	129	0.155	0.232	0.204	0.126
	Phenethyl	2.93	129	0.585	0.602	-0.123	0.495
Phenols	Hydrido	1.51	278	0.155	0.212	0.210	0.010
RC_6H_5OH	4-methyl	2.06	270	0.387	0.456	0.456	0.347
	3-chloro	2.16	318	0.875	0.959	0.959	0.844
	4-ethyl	2.55	269	0.875	0.870	0.972	0.761
	4-propyl	3.04	270	1.273	1.347	1.287	1.260
	2-phenyl	3.28	252	0.909	1.071	0.875	0.897
	4-butyl	3.53	270	1.875	1.814	1.222	1.761
	4-pentyl	4.02	269	1.986	2.115	×	1.921
	4-hexyl	4.52	271	1.260	2.398	×	2.222

Table 4-2. Log(1/MLC) data used for multiple linear regression analysis

		Log(1/MLC) Observed											
Materials	R	*E.coli*			*S.aureus*			*P.aeruginosa*			*E.faecalis*		
Silanols	Methyl	-0.380	-0.391	-0.348	-0.428	-0.375	-0.380	-0.375	-0.371	-0.371	-0.497	-0.505	-0.491
$R(CH_3)_2SiOH$	Vinyl	-0.107	-0.097	-0.064	-0.021	-0.041	0.013	-0.012	0.000	0.013	-0.121	-0.130	-0.114
	Ethyl	-0.017	-0.033	0.000	0.097	0.092	0.108	0.062	0.046	0.081	-0.049	-0.079	-0.041
	n-propyl	0.347	0.367	0.398	0.481	0.456	0.398	0.398	0.432	0.377	0.260	0.301	0.347
	Phenyl	0.602	0.523	0.569	0.602	0.620	0.523	0.523	0.398	0.456	0.398	0.377	0.347
	Benzyl	0.757	0.824	0.770	0.810	0.796	0.757				0.602	0.699	0.602
	n-butyl	0.824	0.903	0.886	0.903	0.886	0.824						
	Phenethyl	1.000	1.097	0.921	1.000	1.155	1.097				0.921	0.886	1.000
Alcohols	Methyl	-1.162	-1.146	-1.083	-1.035	-1.022	-1.021	-0.985	-1.000	-0.986	-1.135	-1.109	-1.130
$R(CH_3)_2COH$	Vinyl	-0.723	-0.719	-0.713	-0.642	-0.634	-0.644	-0.574	-0.556	-0.562	-0.760	-0.734	-0.756
	Ethyl	-0.707	-0.703	-0.709	-0.624	-0.605	-0.629	-0.593	-0.602	-0.597	-0.761	-0.740	-0.760
	n-propyl	-0.236	-0.215	-0.225	-0.236	-0.215	-0.230	-0.013	0.000	-0.021	-0.255	-0.236	-0.243
	Phenyl	0.018	0.046	-0.009	0.102	0.125	0.097	0.158	0.125	0.161	0.018	0.046	0.032
	n-butyl	0.174	0.155	0.187	0.187	0.201	0.171	0.262	0.301	0.276	0.100	0.097	0.155
	Benzyl	0.187	0.155	0.125	0.222	0.244	0.229	0.225	0.201	0.187	0.128	0.097	0.155
	Phenethyl	0.602	0.585	0.569	0.602	0.602	0.569	-0.170	0.000	-0.176	0.483	0.481	0.523
Phenols	Hydrido	0.155	0.149	0.161	0.215	0.201	0.222	0.222	0.260	0.155	0.000	0.009	0.022
RC_6H_5OH	4-methyl	0.398	0.377	0.387	0.456	0.481	0.432	0.523	0.456	0.398	0.301	0.347	0.398
	3-chloro	0.824	0.921	0.886	0.959	0.921	1.000	1.000	0.921	0.959	0.824	0.824	0.886
	4-ethyl	0.824	0.921	0.886	0.921	0.824	0.870	1.000	0.921	1.000	0.770	0.824	0.699
	4-propyl	1.301	1.301	1.222	1.398	1.301	1.347	1.301	1.222	1.347	1.301	1.260	1.222
	2-phenyl	1.000	0.921	0.824	1.155	1.000	1.071	0.824	1.000	0.824	0.824	0.886	1.000
	4-butyl	1.824	1.854	1.959	1.699	1.824	1.959	1.155	1.301	1.222	1.699	1.824	1.770
	4-pentyl	2.000	1.921	2.046	2.222	2.000	2.155				2.000	1.921	1.854
	4-hexyl	1.301	1.222	1.260	2.523	2.301	2.398				2.301	2.222	2.155

Table 4-3. Correlation equations for the antimicrobial activities and the structural properties of the silanols, the alcohols, and the phenols against four bacteria.

Materials	Silanols, Alcohols, and Phenols	
Gram-negative bacteria	*Escherichia coli*	log(1/MLC)=0.7391logP-1.294, n=75, r=0.91, s=0.32, F=352 (4-7) log(1/MLC)=0.63logP+0.0037 Δν-1.84, n=75, r=0.95, s=0.24, F=333, partial F-test=55 (4-8)
	Psedomonas aeruginosa	log(1/MLC)=0.672logP-1.138, n=60, r=0.83, s=0.34, F=128 (4-9) log(1/MLC)=0.55logP+0.004 Δν-1.718, n=60, r=0.94, s=0.23, F=216, partial F-test=95 (4-10)
Gram-positive bacteria	*Staphylococcus aureus*	log(1/MLC)=0.84logP-1.45, n=75, r=0.95, s=0.26, F=676 (4-11) log(1/MLC)=0.743logP+0.0035 Δν-1.971, n=75, r=0.98, s=0.17, F=873, partial F-test=105 (4-12)
	Enterococcus faecalis	log(1/MLC)=0.832logP-1.54, n=72, r=0.95, s=0.26, F=648 (4-13) log(1/MLC)=0.738logP+0.0032 Δν-2.011, n=72, r=0.98, s=0.17, F=837, partial F-test=101 (4-14)
Overall equation		log(1/MLC)=0.786logP-1.379, n=282, r=0.92, s=0.30, F=1543 (4-15) log(1/MLC)=0.679logP+0.0036 Δν -1.909, n=282, r=0.96, s=0.22, F=1639, partial F-test=268 (4-16)

These results revealed that the hydrogen-bonding acidity and the partition coefficient are primary contributors to the antimicrobial activity. The hydrophobicity is an important parameter with respect to the bioactivity such as toxicity or alternation of the membrane integrity because it is directly related to the membrane permeation (McKarns et al., 1997). Hunt (1975) also reported that the potency of aliphatic alcohols was directly related to their lipid solubility through the hydrophobic interaction between the alkyl chain of the alcohols and the lipid region in the membrane. It is reasonable to suggest that a similar hydrophobic interaction may occur between the organic

substituents of silanols or phenols accumulated in the bacterial membrane and the lipid region of the bacterial membranes. As a consequence of the hydrophobic interaction, the bacteria lose their membrane permeability, ultimately causing bacterial death (Hunt, 1975; McKarns et al., 1997).

Kubo pointed out the significance of balance between the hydrophilic and the hydrophobic portions of the molecule for antimicrobial action (Kubo et al., 1993a; Kubo et al., 1993b, 1995). This concept is reasonable because antimicrobials having only a hydrophobic portion but no hydrophilic group or relatively long alkyl chains in their chemical structure showed either reduced or no bioactivities (Tanner, 1943). The hydroxyl function of the alcohols is to orient and preferentially localize the materials near the membrane by virtue of the hydrogen bonding with ester linkages of fatty-acyl residues and with water molecules (Dombek and Ingram, 1984). The hydrogen bonding strength is directly related to the H-bond acidity (West and Baney, 1959). It is reasonable to suggest that the higher H-bond acidity of the silanols compared to the analogous alcohols has contributed to a better balance through the strong hydrogen bonding. A contribution of the H-bond acidity, even though minor, was clear when we compared agents with lower log P and higher H-bond acidity. The higher activity of trimethylsilanol or 4-ethylphenol when compared to that of ethyldimethylcarbinol or n-butyldimethylsilanol respectively appears to be attributed to their higher H-bond acidity as described in Chapter 3. It is reasonable to speculate that the silanols may disrupt the cell membrane more efficiently than the analogous alcohols due not only to their higher hydrophobicity but also the higher H-bond acidity.

Hansch reported a number of correlation equations involving antimicrobial activity with alcohols and phenols (Lien et al., 1968). For benzyl alcohols against *Escherichia coli* a log (1/C) = 0.539 log P +0.531σ +4.001, n=14, r=0.939 was reported. For *Staphylococcus aureus* and *Enterococcus faecalis* a log (1/C) = 0.599 log P + 0.421σ + 4.069, n=18, r=0.906 was observed. Substituted phenols tested against *Psedomonas aeruginosa* displayed a log (1/C) =0.684 log P - 0.921 σ +0.265, n=21, r=0.847 (Lien et al., 1968). For the electronic or polar property Hansch used the Hammitt σ constant, whereas the H-bond acidity was employed in this study. The value of the Hammitt σ constant is dependent on the electronic properties of the substituent X relative to the substituent H in aromatic compound. The H-bond acidity employed depends not only on the electronic property of the substituents but also the parent molecules. As a result, the correlations obtained from the previous studies were limited to a homologous series of chemicals such as substituted phenols or benzyl alcohols individually and did not allow for combining different series of chemicals. This is first time that the silanols, the alcohols, and the phenols were studied as one group of chemicals. It is therefore significant that a linear relationship was found between the antimicrobial activities and the lipophilicity and the H-bond acidity of the antimicrobials.

4.3.2 Statistical Methods

The significance and validity of the regression equations could be evaluated by assessing the correlation coefficient r, the standard deviation s, and the F values (Kubinyi, 1993; Livingstone, 1995). The correlation coefficient r is a measure of how well the predicted values from a model fit with the actual data. The data is significant if the correlation coefficient r is approximately or higher than 0.9 for in vitro data and approximately 0.8 for whole animal data. The standard deviation s is an absolute measure

of the quality of the fit. If the standard deviation s is not much larger than the standard deviation of the biological data, normally around 0.3, the model is acceptable. The F value is a measure of the statistical significance of the regression model. If the F value is larger than the 95% significance limits shown in Table 4-4 the model can be considered statistically significant (Livingstone, 1995).

The correlation coefficients and standard deviations for one parameter models were r = 0.91 and s= 0.32 for *Escherichia coli*, r = 0.95 and s=0.26 for *Staphylococcus aureus*, r= 0.83 and s= 0.34 for *Psedomonas aeruginosa*, and r=0.95 and s=0.26 for *Enterococcus faecalis*. It appears that the correlation coefficients and the standard deviation values achieved are significant enough to prove that the lipophilicity is the primary contributor for antimicrobial activity except for *Psedomonas aeruginosa*. In the case of the two parameters models, an improvement of the correlation coefficient, i.e., 0.91 to 0.95 for *Escherichia coli*, 0.83 to 0.94 for *Psedomonas aeruginosa*, 0.95 to 0.98 for *Staphylcoccus aureus*, and 0.95 to 0.98 for *Enterococcus faecalis*, with lower value of the standard deviations than those of one parameter models were observed. Two different regression models containing different numbers of independent variables can be compared by a partial F test value calculated by Equation 4-18. The significance of introducing a new variable can be justified if the partial F value is larger than 95% significance levels presented in Table 4-4. The F values calculated with Equation 4-17 for each model were greater than those of the 95% confidence levels, which verify that the models are statistically significant. Addition of the H-bond acidity parameter to the one parameter model with the lipophilicity was found to be significant based on the calculated partial F values presented in Table 4-3.

$$F = \frac{r^2(n-k-1)}{(k(1-r^2))} \tag{4-17}$$

$$\text{Partial } F = \frac{(r_2^2 - r_1^2)(n-k_2-1)}{(k_2-k_1)(1-r_2^2)} \tag{4-18}$$

Where n is the number of data, k is the number of variables, k_2 is the larger number of variables, k_1 is the smaller number of variables, r_2 is the correlation coefficient for the larger number of variables, and r_1 is the correlation coefficient for the smaller number of variables.

Table 4-4. Selected F values (DF=n-k-1); two sided, 95% significance level, n is the number of data, k is the number of variables. (Kubinyi, 1993; Livingstone, 1995)

DF	k		
	1	2	3
20	4.35	3.49	3.10
30	4.17	3.32	2.92
60	4.00	3.15	2.76

4.3.3 Variation of the Cut-off Points

It has been previously reported that the antimicrobial activity of the aliphatic alcohols falls off as the alkyl chain increases to more than six carbon atoms (Rotter, 1984). This is called the cut-off point where the biological activity falls rapidly or disappears as the chain length increases in a homologous series. In the case of the twenty-five antimicrobial agents, the silanols, the alcohols, and the phenols against four different bacteria, the cut-off points varied with bacteria. In the case of the Gram-negative bacterium, *Escherichia coli*, a clear cut-off point of antimicrobial activity can be seen in Figure 4-1(A). The activity began to decrease as the partition coefficient increase beyond a certain point. In contrast, no cut off point was detected for *Staphylococcus aureus* as

shown in Figure 4-1(B). The disappearance of the antimicrobial activity at the cut-off point was also detected for *Psedomonas aeruginosa* and *Enterococcus faecalis* as shown in Figure 4-2. For *Psedomonas aeruginosa* minimum lethal concentration values of n-butyl-, benzyl-, phenethyldimethylsilanol and 4-pentyl-, 4-hexyl phenol were not detected under the antimicrobial activity test conditions described in Chapter 3. Benzyldimethylcarbinol showed a reduced activity when compared to butyldimethylcarbinol as well as phenethyldimethylcarbinol exhibited a lower activity than benzyldimethylcarbinol against *Psedomanoas aeruginosa* as demonstrated in Figure 4-2(A). n-butyldimethylsilanol did not show a 7-log reduction against *Enterococcus faecalis*. It was found that n-butyldimethylsilanol required more than an hour to show a 7-log reduction against *Enterococcus faecalis*. It should be mentioned that all of the cut-off points were observed within the experimental condition of 1 hour exposure time at the given concentration of bacteria in the aqueous solution. In other words, the cut-off point can vary depending on the exposure time and bacterial concentration or medium used.

There are several suggestions explaining the reason for the occurrence of a cut-off point and how is varied with bacteria (Hansch and Fujita, 1964; Lien et al., 1968). Hansch claimed that it is related to the rate of diffusion of the molecule into the cell. The rate depends upon how strongly molecules are bound by the protein or lipid they interact with in the membrane section. Hansch et al. suggested that the fall off in activity with increase in hydrophobicity was due to a slow diffusion rate of molecules which are strongly bound in the membrane. Consequently, it is not possible to accumulate a sufficient concentration at the reaction site such as the membrane to produce the particular biological response within a test period. Additionally, Ferguson suggested that

the limiting solubility of the materials in the hydrophilic phase led to the fall-off of the activity (Burger, 1960; Ferguson, 1939; Hansch and Fujita, 1964). Drug molecules will not be able to cross the aqueous phase barrier of the membrane when the lipophilicity of the drug reaches a point where a micelle begins to form or when the drug molecules are localized in the first lipophilic phase of the membrane.

Escherichia coli and *Psedomonas aeruginosa*, Gram-negative bacteria, showed lower cut off points than those of gram-positive bacteria, *Staphylococcus aureus* and *Enterococcus faecalis*, similar to previous studies (Klarmann, 1932; Lien et al., 1968). For 4-hexyl phenol, the Gram-positive bacteria did not exhibit the fall-off whereas the Gram-negative bacteria with the same substituents displayed either reduction or disappearance of antimicrobial activity as shown Figure 4-1 and 4-2. Klarmann (1932) reported that for the Gram-negative bacteria, the maximum activity was reached at the amyl derivative of chloro-phenol against *Eberthella typhi* and the hexyl derivative against *Eberthella paradysenteriae*. However, in the case of the Gram-positive bacterium, *Staphylococcus aureus*, maximum activity with the *n*-octyl derivative was observed. Hansch also reported that Gram-positive bacteria showed a higher cut-off point with an optimum partition coefficient value of 6, than that of Gram-negative bacteria with a value of 4 (Lien et al., 1968). These authors claimed that the lower cut –off point with the Gram-negative bacteria was due to their higher lipid content contributing to a higher bacterial resistance. We speculated that the outer membrane, the selective permeability barrier of the cell wall in Gram-negative bacteria exclude big molecules from penetrating into the cell. The hydrophilic small molecules up to a molecular weight of 650-800 daltons can diffuse through the porins. The porins are proteins described as water-filled

channels in the outer membrane that form pores, wide enough to allow passage of small hydrophilic molecules, but hydrophobic molecules can be excluded. The hydrophobic or amphipilic molecules, depending on their size, can diffuse through the bilayers composed of phospholipids and lipopolysaccaride. In contrast, the cell wall of Gram-positive bacteria is composed of a thick peptidoglycan without a permeability barrier. The peptidoglycan can be considered as a strong or rigid, woven mesh that holds the cell shape and prevents osmotic lysis. The openings in the mesh are large enough for big molecules to pass through it (Atlas, 1984; Barton, 2005).

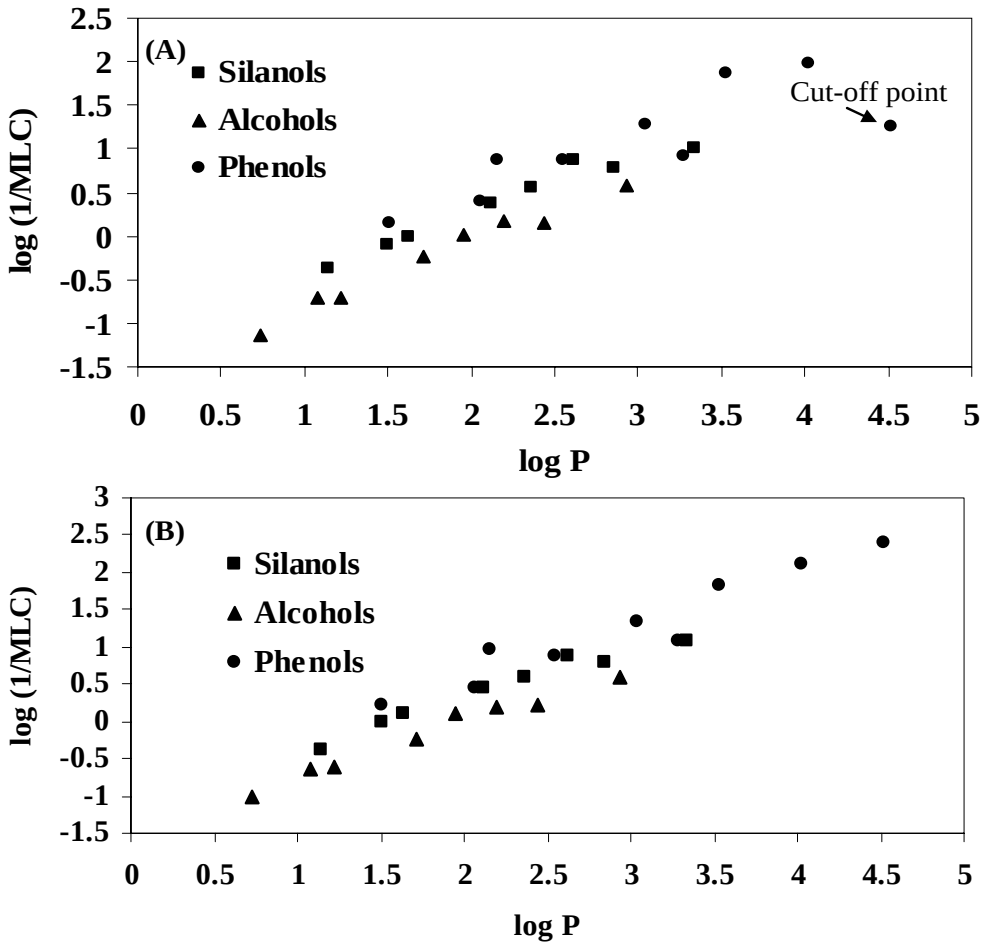


Figure 4-1. Correlation between antimicrobial activities and the partition coefficient, for (A) *Escherichia coli* and (B) *Staphylococcus aureus*. Data points are from Table 4-1.

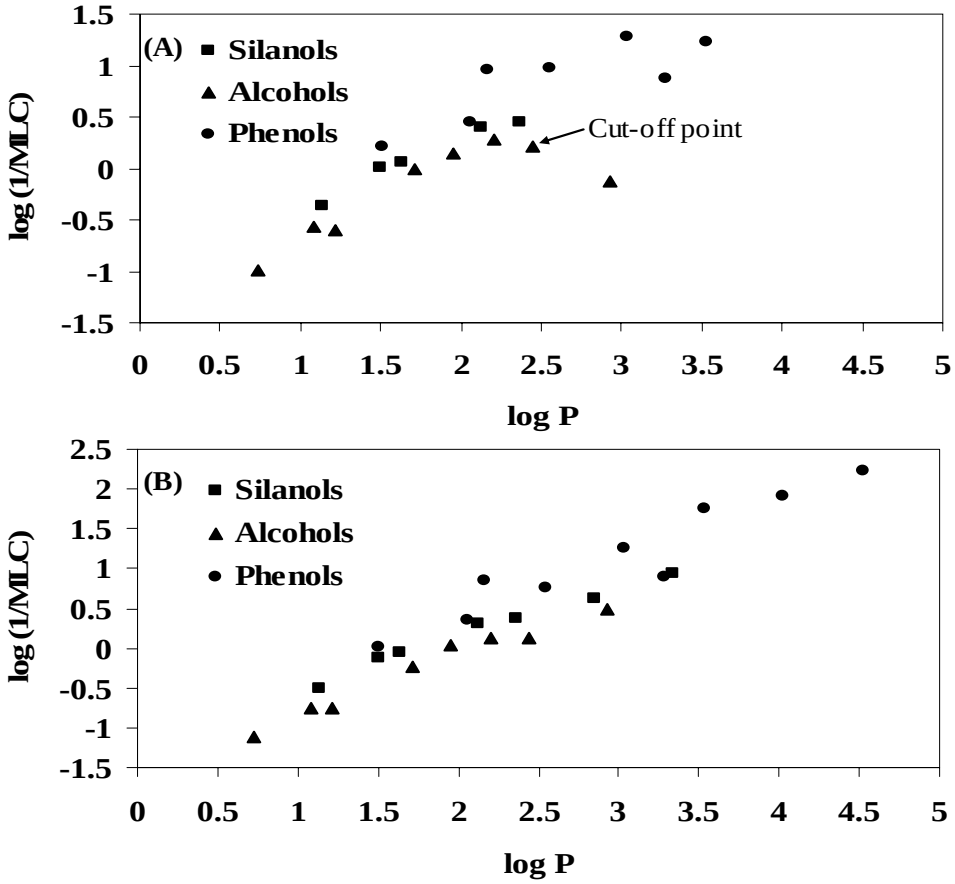


Figure 4-2. Correlation between antimicrobial activities and the partition coefficient, for (A) *Pseudomonas aeruginosa* and (B) *Enterococcus faecalis*. Data points are from Table 4-1.

4.3.4 Prediction of External Compounds

The predictive ability of the models was evaluated by testing structurally diverse compounds called external compounds that were not used in the creation of the correlation equations. A difference between the observed data and the predicted data of the external compounds can be a measure of the predictive ability of the correlation models. The widely used measures are the prediction error sum of squares (PRESS) and root mean square prediction error (RMSPE) (Stone and Jonathan, 1993).

$$PRESS = \sum_i (y_{e,i} - y_{p,i})^2 \qquad (4\text{-}19)$$

$$RMSPE = \sqrt{\frac{PRESS}{n}} \quad (4\text{-}20)$$

Where $y_{e,i}$ are experimental values and $y_{p,i}$ are predicted values, n is number of data.

Four classes of external samples from different structural feature were tested. Tertiary structure of silanols and alcohol are triethylsilanol, 3,3,3-trifluoropropyldimethylsilanol, cyclohexyldimethylsilanol, and pentyldimethylcarbinol. *n*-primary structure alcohols are n-hexanol, n-heptanol, n-octanol. Diol structures are 1,2 octanediol and disiloxanediol. Di-substituted phenols are thymol ((5-methyl-2-(1-methylethyl)phenol) and 4-chloro-3-methyl phenols. The structural properties of the external compounds are summarized in Table 4-5 as well as their measured minimum lethal concentrations along with calculated MLCs. Calculated log (1/MLC) was plotted against observed log (1/MLC) as shown in Figure 4-3 for *Escherichia coli* test and Figure 4-4 for *Staphylococcus aureus* to determine which group of external compounds is fit better for the correlation equations. Triethylsilanol and thymol appear to be well predicted for both *Escherichia coli* and *Staphylococcus aureus*. Pentyldimethylcarbinol, 4-chloro-3-methyl phenol, and cyclohexyldimethylsilanol were moderately matched with the calculated MLCs as reported in Table 4-5. In contrast n-primary alcohols, 3,3,3-trifluoropropyldimethylsilanol, and diols were relatively poorly predicted.

Considering the structural features, compounds with the tertiary structure and the disubstituted phenols were found to be relatively well predicted based on the comparison data of the RMSPE presented in Table 4-6 and Figure 4-3 and 4-4. The good prediction should be a result of the resemblance in the chemical structures because tertiary structure compounds were employed for the models whereas n-primary and diols structures were not used in the development of the correlation models. Branching of alkyl chain was

found to affect antimicrobial activity in the order of *n*-primary>secondary> tertiary alcohols (Ali, 2001; Tanner, 1943). Minimum effective concentrations required for n-primary alcohols such as *n*-butanol or *n*-pentanol was about three times lower than that of tertiary alcohols even though their calculated partition coefficient was somewhat similar (Ali, 2001). Measured MLC of 1, 2- octanediol was much lower than that of predicted. We suspect that it is due to an n-primary structure even though it has two hydroxyl groups. Addition of hydroxyl group was known to be a potential factor for lowering antimicrobial activity (Suter, 1941). For disiloxanediol the effect of increasing the number of hydroxyl group was observed by acquiring higher concentration of the agents, 4.75 (% g/g) than that predicted, 2.16-0.87 (% g/g). The predictive ability of the correlation models seems to be dependant on the chemical structures. An evaluation with more external compounds is recommended to obtain a better understanding or assessment of the predictive ability of the models.

Table 4-5. Comparison of the minimum lethal concentration (MLC) between observed MLC and calculated MLC from the equations in Table 4-3.

			Escherichia coli			*Staphylococcus aureus*		
			Observed	Calculated		Observed	Calculated	
External compounds	log P	Δv	MLC	MLC1	MLC2	MLC	MLC3	MLC4
Triethylsilanol	2.62	231	0.19	0.228	0.217	0.16	0.178	0.166
3,3,3-trifluoropropyl dimethylsilanol	2.54	274	0.65	0.261	0.169	0.55	0.207	0.133
Cyclohexyl dimethylsilanol	3.41	230	0.13	0.059	0.07	0.055	0.039	0.043
Pentyldimethyl carbinol	2.69	124	0.19	0.202	0.488	0.325	0.155	0.347
n-hexanol	1.82	144	0.375	0.889	1.452	0.425	0.834	1.307
n-heptanol	2.31	144	0.175	0.386	0.713	0.150	0.323	0.565
n-octanol	2.81	143	0.045	0.165	0.345	0.053	0.123	0.240
1,2-octanediol	1.67	177	0.325	1.148	1.353	0.325	1.115	1.286
Disiloxanediol	1.26	298	4.750	2.306	0.878			
Thymol	3.52	272	0.048	0.049	0.041	0.048	0.031	0.025
4-chloro-3-methyl phenol	2.70	298	0.068	0.199	0.109	0.063	0.152	0.083

Table 4-6. Comparison data of PRESS and RMSPE of different classes of external compounds against *Escherichia coli* and *Staphylococcus aureus*

	Escherichia coli				*Staphylococcus aureus*			
	Predicted values $y_{p,i}$ - MLC1		Predicted values $y_{p,i}$ - MLC2		Predicted values $y_{p,i}$ - MLC3		Predicted values $y_{p,i}$ - MLC4	
External compounds	PRESS	RMSPE	PRESS	RMSPE	PRESS	RMSPE	PRESS	RMSPE
Tertiary structure	0.16	0.20	0.32	0.28	0.15	0.20	0.17	0.21
n-primary structure	0.32	0.33	1.54	0.71	0.2	0.26	0.98	0.57
Disubstituted phenols	0.017	0.092	0.002	0.03	0.008	0.064	0.001	0.021
Diol structure	6.65	1.82	16.05	2.83	0.62	0.79	0.92	0.96

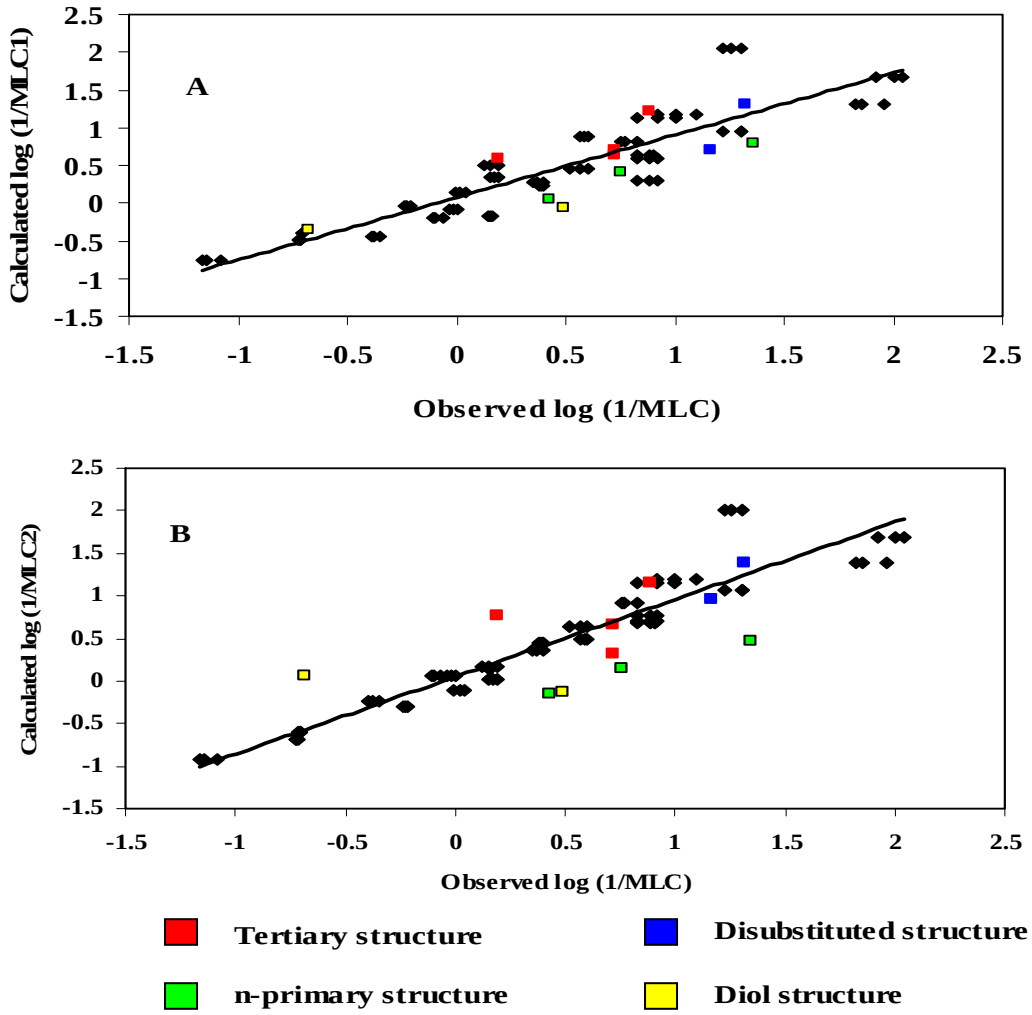


Figure 4-3. Comparison between the external compounds and the silanols, alcohols, and phenols against *Escherichia coli*. A is for MLC1 calculated from the one parameter equation, B is for MLC2 calculated from the two parameter equation.

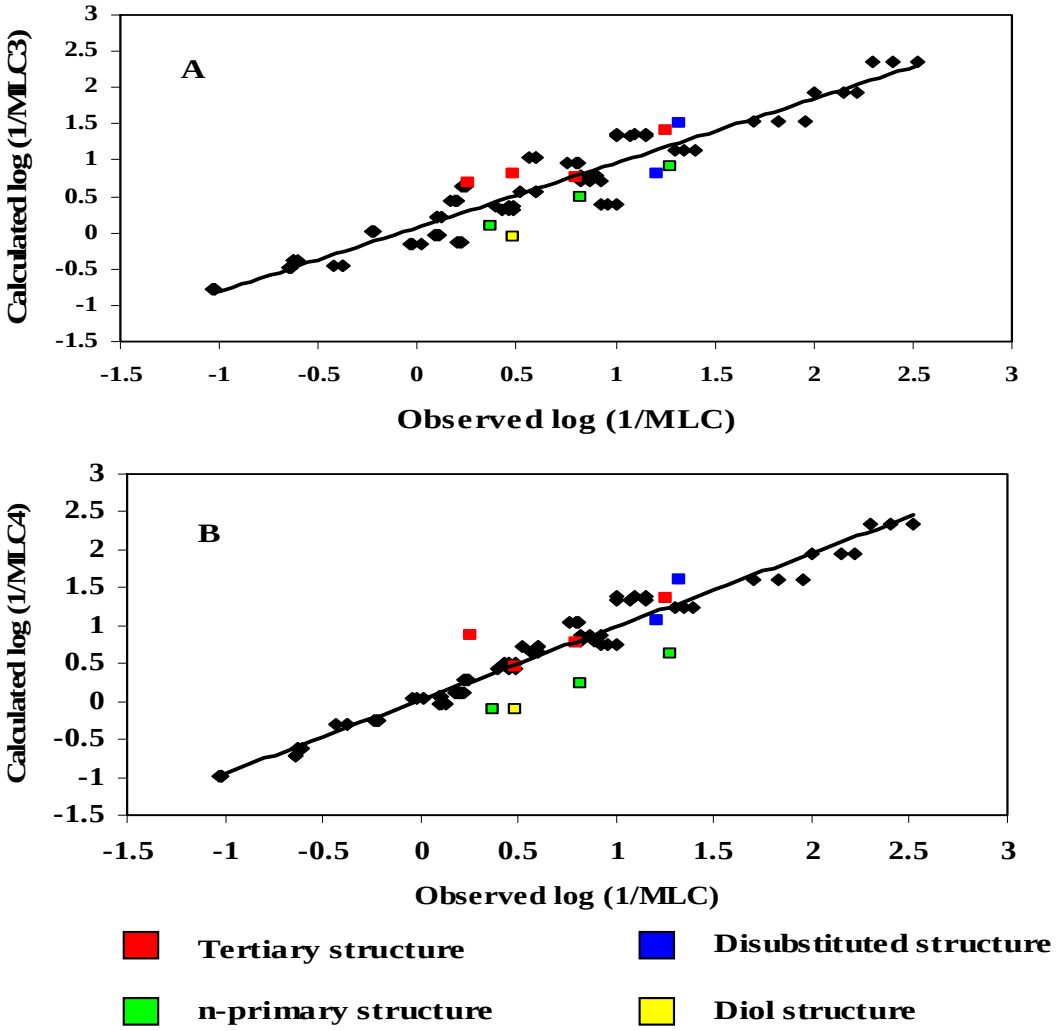


Figure 4-4. Comparison between the external compounds and the silanols, alcohols, and phenols against *Staphylococcus aureus*. A is for MLC3 calculated from the one parameter equation, B is for MLC4 calculated from the two parameter equation.

In Table 4-5 and 4-6 and Figure 4-3 and 4-4, equations used for calculation of MLC values are; MLC1 is calculated from $\log(1/MLC1)=0.739\log P-1.294$, MLC2 is estimated from $\log(1/MLC2)=0.63\log P+0.0037\ \Delta v-1.84$, MLC3 is calculated from $\log(1/MLC3)=0.84\log P-1.45$, and MLC 4 is obtained from $\log(1/MLC4)=0.743\log P+0.0035\ \Delta v-1.971$.

4.5 Conclusions

A structure-activity dependence study with four bacteria was carried out to determine the relationship between structural properties of silanols, alcohols, and phenols and their antimicrobial activities. In this study, silanols, alcohols, and phenols were treated as belonging to the same group of chemicals which have the hydrophobic regions and the hydrophilic portion containing hydroxyl group. The antimicrobial activity of the chemical compounds was found to be closely related to their lipophility and H-bond acidity. The empirical models suggested that the antimicrobial activity increases as their lipophilicity increases. However, there was a cut off point where the activity begins to decrease as the lipophilicity continuously increases. The effect of the H-bond acidity to the antimicrobial activity was relatively small, showing that the activity was enhanced with higher H-bond acidity. The outcome of the empirical equations corresponding to the four bacteria, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis* was similar, indicating that the kill mechanisms of those bacteria may be resemble as well. The cut-off points found appear to vary with tested bacteria.

CHAPTER 5
CHARACTERIZATION OF MEMBRANE DAMAGE OF BACTERIA CAUSED BY SILANOLS

5.1 Introduction

The silanols, the alcohols, and the phenols have a similar chemical structure consisting of a hydrophilic hydroxyl group and a hydrophobic organic substitutent. Alcohols and phenols are widely used antimicrobial agents while the silanols were recently discovered to be a biocidal agent (Kim et al., 2006). It is plausible to predict that the antimicrobial mode of actions of the silanols may resemble those of the alcohols and the phenols. It is generally known that alcohols cause membrane damage (Dombek and Ingram, 1984; Lucchini et al., 1993; Lucchini et al., 1990) through an increase of the lipid solubility. In other words, alcohols increase the membrane fluidity through disorder of the biological self-assembly of the membrane. Denyer (1990) reported that the primary target site of the phenolic compounds is the cytoplasmic membrane. The damage to the cytoplasmic membrane causes the membrane to lose its ability to be a permeability barrier, subsequently leading to a loss of structural integrity and a leakage of intracellular material.

A bacterium has several layers of material that enclose or protect its cytoplasm (Atlas, 1984, 1986; Barton, 2005). The cytoplasm region contains critical life units such as the cell genome (DNA), the ribosomes, and various sorts of inclusions. These critical life units are protected by the plasma membrane, the cell wall and the capsule as seen in Figure 3-1. The cell wall is an essential part that protects the cytoplasm from mechanical

damage and from osmotic rupture or lysis. In the Gram-positive bacteria, such as *Staphylococcus aureus* and *Enterococcus faecalis,* the cell wall is thick (15-80 nanometers) consisting of several layers of peptidoglycan. On the other hand, Gram-negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa* have relatively thin (10 nanometers) cell wall composed of a single layer of peptidoglycan and surrounded by an outer membrane as seen in Figure 5-1.

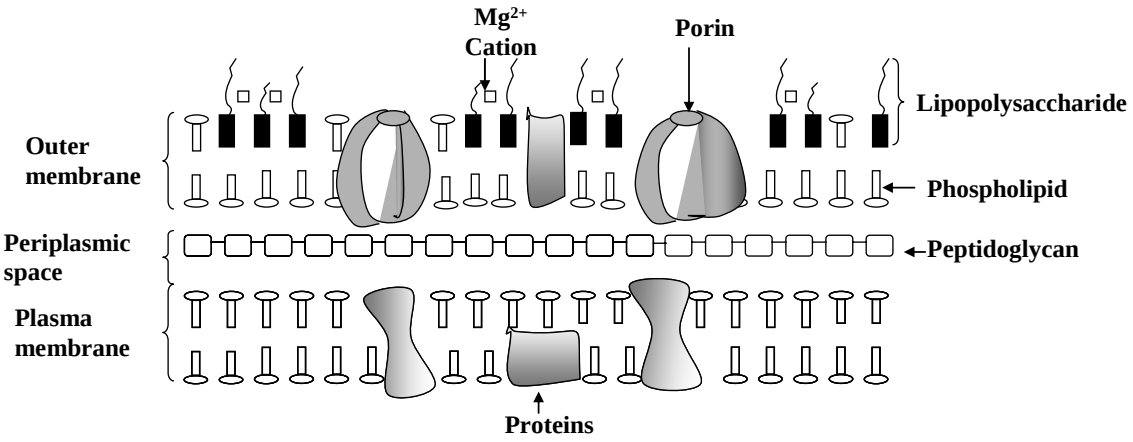


Figure 5-1. Schematic illustration of the outer membrane, peptidoglycan, and the plasma membrane of a Gram-negative bacterium (*Escherichia coli*).

The outer membrane is a bilayered structure with phospholipids, lipopolysaccharide, proteins, and ions. For Gram-negative bacteria, the outer membrane is the first permeability barrier. The plasma membrane, located underneath of the cell wall and called the cytoplasmic membrane, is a selective permeability barrier that regulates the passage of substances into and out of the cell. The plasma membrane is the last barrier that can separate or protect the cytoplasm from the environment (Atlas, 1984, 1986; Barton, 2005). Bacteria membranes are composed of a phospholipid bilayer combined with a variety of proteins and ions. The phospholipids are amphoteric molecules with a polar hydrophilic head and two non-polar hydrophobic fatty acid tails, which naturally form a bilayer in aqueous environments. Both electrostatic and

hydrophobic interactions are involved in maintaining the spatial organization of the membrane components (Ingram and Buttke, 1984). Disorganization of the membrane by undesired or foreign substances can cause loss of the permeability or of the integrity of the membrane, consequently causing the death of the cell.

For many antimicrobial agents, antimicrobial actions are initiated by interactions of the biocides with the cell wall membrane of the microorganisms. The agents then penetrate into the cell, and finally act at the target sites (McDonnell and Russell, 1999). Hunt (1975) proposed that the potency of aliphatic alcohols is directly related to their lipid solubility. The lipid in the membrane bilayers can be dissolved by alcohols through the induced hydrophobic interaction between the alkyl chain from the alcohols and the lipid region in the membrane (Hunt, 1975; Ingram and Buttke, 1984). The hydrophobicity is an important parameter with respect to toxicity or alternation of the membrane's integrity because it is directly related to the membrane permeation which can result in disruption of the membranes of the bacterium. A plasma membrane integrity test with short-chain aliphatic alcohols reported in the literature illustrated that an increase of the alkyl chain length from methanol to 1-octanol enhanced the loss of the membrane integrity of epithelial cells in vitro (McKarns et al., 1997). Those authors suggested that alteration of the membrane integrity correlates with the hydrophobic properties of the alcohols.

In this study we suggest that the hydrophobic interaction occurs between the organic group of the silanols and the lipophilic portion of the membranes as well as the hydrogen bonding between the hydroxyl function of the silanol and either the ester linkages of fatty-acyl residues or water molecules (Ingram and Buttke, 1984). We

speculate that the silanol can partition effectively into the membrane through these interactions and cause its disorganization by increasing lipid solubility. Ultimately the membrane damage results in the death of the bacteria. Transmission electron microscopy and fluorescent dye techniques were utilized to demonstrate the membrane damage on the bacteria treated by triethylsilanols.

5.2 Experiment

5.2.1 TEM Sample Preparation Procedures

Bacterial suspensions, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Enterococcus faecalis*, were treated by triethylsilanol at a concentration of 0.2 (% g/g) for 1 hour. The control was prepared from the same batch of bacterial suspension at the same condition as the treated one. The plate count method was used to evaluate the viability of the tested bacteria after the treatment. No bacteria growth was observed after the triethylsilanol treatment. The control bacterial suspension and the treated bacteria suspension were centrifuged and washed with a buffer solution 0.1M sodium cacodylate, pH 7.2-7.4, followed by centrifugation at 2600rpm for 5 minutes to concentrate the bacteria cells in a pellet. Primary fixation of the bacteria consisted of a 1:1 mixture of a 1% solution of OsO_4 containing 7.5mg of potassium ferricyanide/ml and 5% of glutaraldehyde in the buffer was prepared (Hasty and Hay, 1978). 5ml of the primary fixation solution was added to the bacteria, were fixed for 1hour, followed by centrifugation and washed with the buffer 2 times for 10 minutes. Bacterial suspensions were transferred to 0.5ml microtubes for ultracentrifugation and removal of supernatant, followed by addition of 3% Type VII low gelling temperature agarose for bacteria encapsulation. The samples were washed 3 times for 10 minutes with deionized water. An ethanol graded dehydration series, 25%, 50%, 75%, 90%, and 100%, was used to

dehydrate the sample for 20 minutes, followed by 100% acetone for 15 minutes. The samples were infiltrated with different concentrations of Embed 812 epoxy resin diluted in acetone, 30%, 50%, 70%, and 100% respectively, for 1 hour, then polymerized in an oven at 75C^{o} for 24hours. Ultra thin sections (70nm) were obtained by microtome and placed on 400 mesh uncoated copper grids. Post staining was done with 2%uranyl acetate and Reynold's lead citrate. The samples were examined and photographed at an accelerating voltage of 75kV using a Hitachi H-7000 transmission electron microscopy at Electron Microscopy Core Laboratory at the University of Florida..

5.2.2 Fluorescent Dye Studies

Fluorescent dye, LIVE/DEAD BacLight Bacterial Viability Kits, provided by Molecular probes has a capability of monitoring the viability of the bacteria as a function of the cell's membrane integrity (Bunthof et al., 2001; Laflamme et al., 2004). Bacteria with a compromised membrane that are considered to be dead or dying will stain red, whereas cells with an intact membrane will stain green. The LIVE/DEAD BacLight Bacterial Viability Kit L7007 is composed of SYTO 9 green-fluorescent nucleic acid stain and the red-fluorescent nucleic acid stain, Propidium iodide. The red and green stain show difference in spectral characteristics, different emission wavelength, and in their ability to penetrate healthy bacterial cells. The green stain can label bacteria with intact membranes and with damage membranes. In contrast, the red stain, Propidium iodide, penetrates only bacteria with damaged membranes and causes a reduction in the green fluorescence when the both dyes are present. The excitation /emission range of the green stain is 470/510-540nm and 470/620-650nm for the red stain. Fluorescence microscopy and fluorescence spectroscopy methods can be used to evaluate the viability of the bacteria as a function of the membrane integrity.

Materials and methods. Four different bacterial suspensions, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus,* and *Enterococcus faecalis*, in deionized water were prepared based on the method described in Chapter 3. The bacteria suspensions were treated by triethylsilanol and phenethyldimethylsilanol using the minimum lethal concentrations of each material for 1 hour. Viabilities of treated bacteria were confirmed by the plate-count method (Collines, 1995). The fluorescent dye kit has the green stain, 3.34mM in DMSO, and the red stain, 20mM in DMSO. Equal volumes of the red and the green dye are combined in a microfuge tube and mixed thoroughly for one minute. 3µl of the dye mixture was added into each 1ml of the bacterial suspension, mixed thoroughly, and kept in the bark for about 15minutes.

Fluorescence microscopy. 5µl of the stained bacterial suspension was placed between a slide and a cover slip for microscopy evaluation. MRC-1024 confocal laser scanning microscopy at the McKnight Brain Institute at the University of Florida was used for observation of the stained bacteria at 40X (magnification). The LaserSharp software can deliver simultaneous three channels acquisition and display. The channels for red dye and the green dye acquired the picture and displayed simultaneously, then, the combined picture of these two dyes also was displayed as a result.

Fluorescence spectroscopy. One milliliter of the stained bacterial suspension was placed in a quartz rectangular cell for spectrophotometer. The cell has Suprasil quartz windows with a 10mm lightpath. The fluorescence emission was measured at the emission spectrum (excitation 470nm, emission 500-700nm) by a Perkin-Elmer MPF-44B fluorescence spectrophotometer at Particle Engineering Research Center at the University of Florida.

5.3 Results and Discussion

5.3.1 A Study of Membrane Damage of Bacteria by TEM

Transmission electron microscopy revealed structural membrane damage for the four bacteria, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Enterococcus faecalis*, treated by triethylsilanol for 1 hour at the minimum lethal concentration of 0.2%. The smooth continuous membrane structure was clearly observed in the untreated bacteria while the treated bacteria showed relatively disorganized membrane structures. The Gram-negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa*, have an outer membrane and a plasma membrane, whereas the Gram-positive bacteria, *Staphylococcus aureus* and *Enterococcus faecalis*, have only a plasma membrane and a thick cell wall. Primary fixation allows the membrane structures to be preserved, glutaraldehyde crosslinks the proteins and OsO_4 crosslinks the lipids. Since triethylsilanol treated bacteria and control cell were processed with the same procedure, the differences observed was likely caused by the silanol treatment.

The membranes were clearly identified in the TEM pictures due to the primary fixation of OsO_4 and the post staining of the bacteria. The dark areas show where the sample had a high electron density and the light areas show where the sample had a low electron density. For *Escherichia coli* treated by triethylsilanol the cytoplasma membrane appeared to be retracted from the outer membrane and disorganized whereas the control showed an intact cell envelope between the outer membrane and the cytoplasma membrane as shown in Figure 5-2. Corre and his associates (1990) reported that a membrane alteration of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* occurred with a phenethyl alcohol treatment. These authors claimed that the Gram-negative cell envelops were permeabilized and the plasmic membrane in

Staphylococcus aureus was solubilized with phenethyl alcohol treatment. The cell wall dissociation from the cytoplasmic membrane of *Escherichia coli* was reported as well as enlargement of the cell wall of *Staphylococcus aureus* upon phenethyl alcohol treatment (Corre et al., 1990). In Gram-positive bacteria, *Eentrococcus faecalis* and *Staphylococcus aureus*, the cytoplasma membrane disruptions are clearly visible in Figure 5-3 and 5-4 respectively. The cytoplasma membrane lost its integrity and showed disordered structures when compared to the control which shows a smooth and intact membrane. Unlike the result from the phenethyl alcohol treatment on *Staphylococcus aureus* (Corre et al., 1990) the enlargement of the cell wall of the Gram-positive bacteria are not noticeable for the triethylsilanol treated bacteria. Cross wall formation was observed in the Gram-positive bacteria, indicative of cell division. The silanol treated *Staphylococcus aureus* also showed the cytoplasm region that appears to be inhomogeneous when compared to the control. The inhomogeneous appearance of the cytoplasm might be a result of segregation of the internal organelles. A rough surface on the outer membrane structure was detected on treated *Psedomonas aeruginosa* when compared to the smooth and continuous outer membrane of the untreated bacterium in Figure 5-5. However, a difference between the treated and the control for *Psedomonas aeruginosa* was relatively insignificant. A loss of integrity of the membranes may lead to malfunction of the permeability barrier, followed by osmotic rupture or lysis of the cytoplasma. Ultimately the bacteria lose their viability due to the series of actions taking place after the membrane damage.

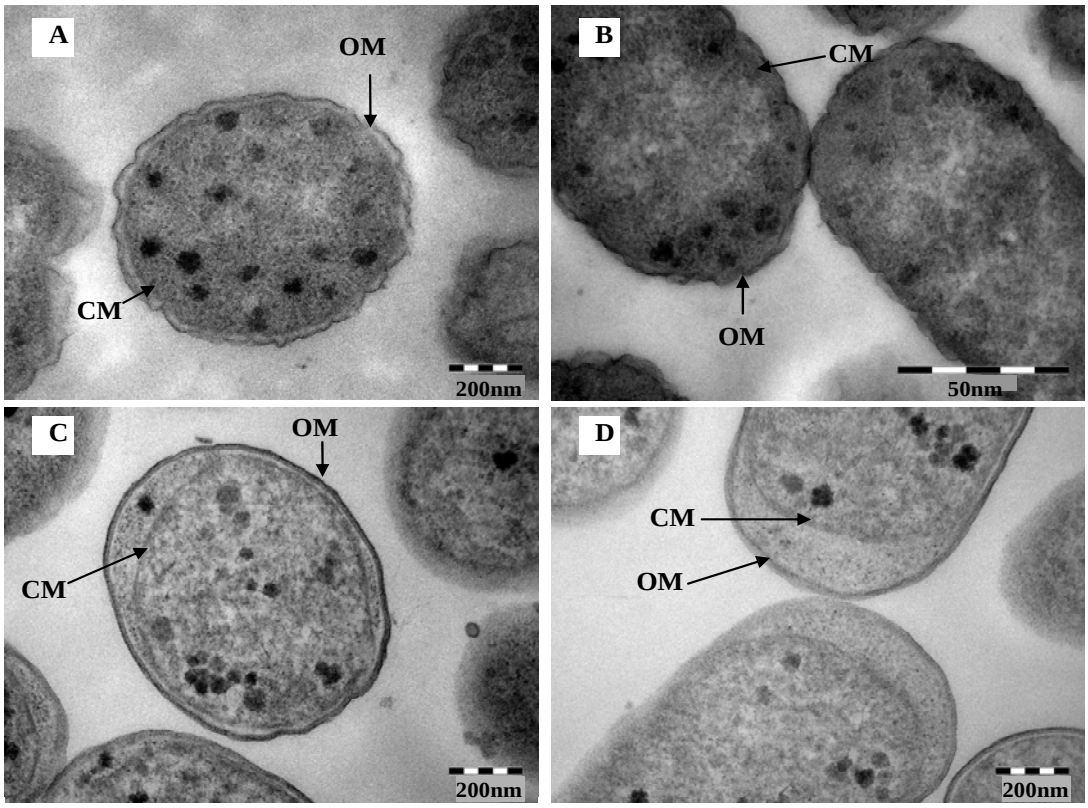


Figure 5-2. *Escherichia coli* TEM pictures- A and B are the control samples showing intact cell envelope. C and D are triethylsilanol treated with detachment of the CM (cytoplasmic membrane) from the OM (outer membrane)

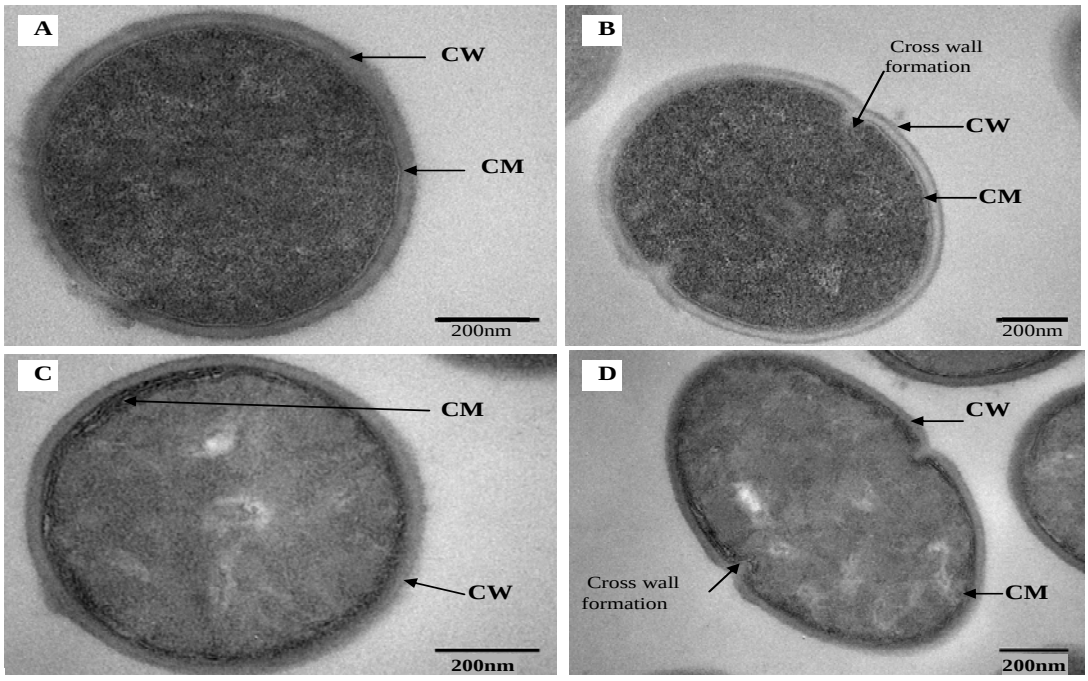


Figure 5-3. *Enterococcus faecalis* TEM pictures- A and B are the control samples. C and D are triethylsilanol treated samples with the disrupted CM (cytoplasmic membrane) and CW (cell wall)

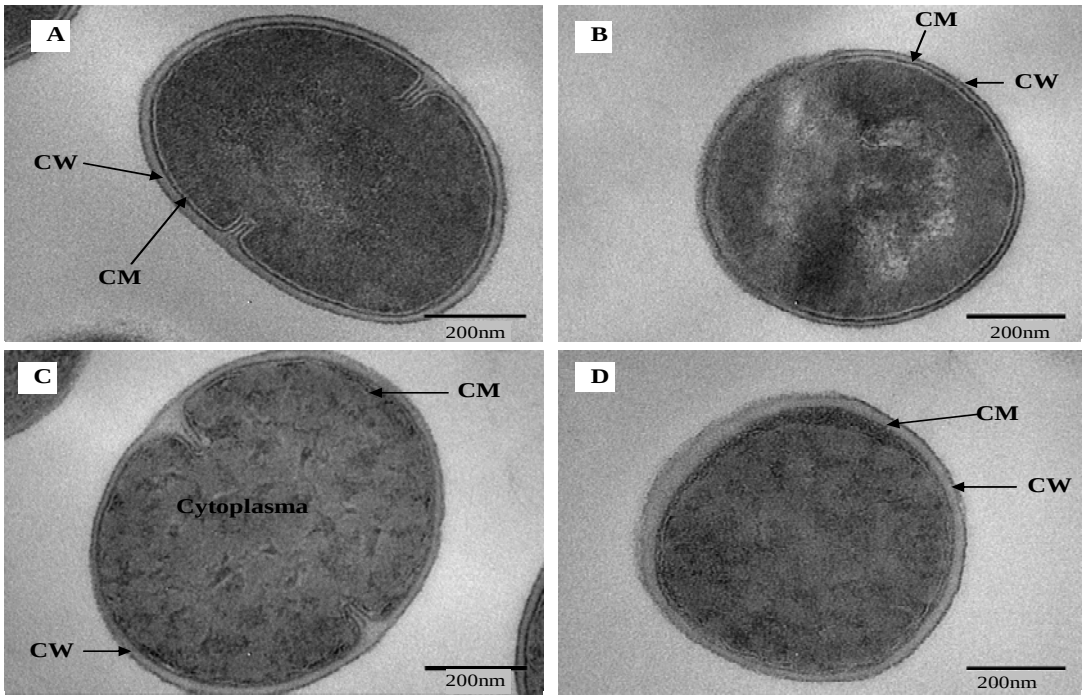


Figure 5-4. *Staphylococcus aureus* TEM pictures- A and B are the control samples. C and D are triethylsilanol treated samples with the disorganized CM (cytoplasmic membrane) and CW (cell wall)

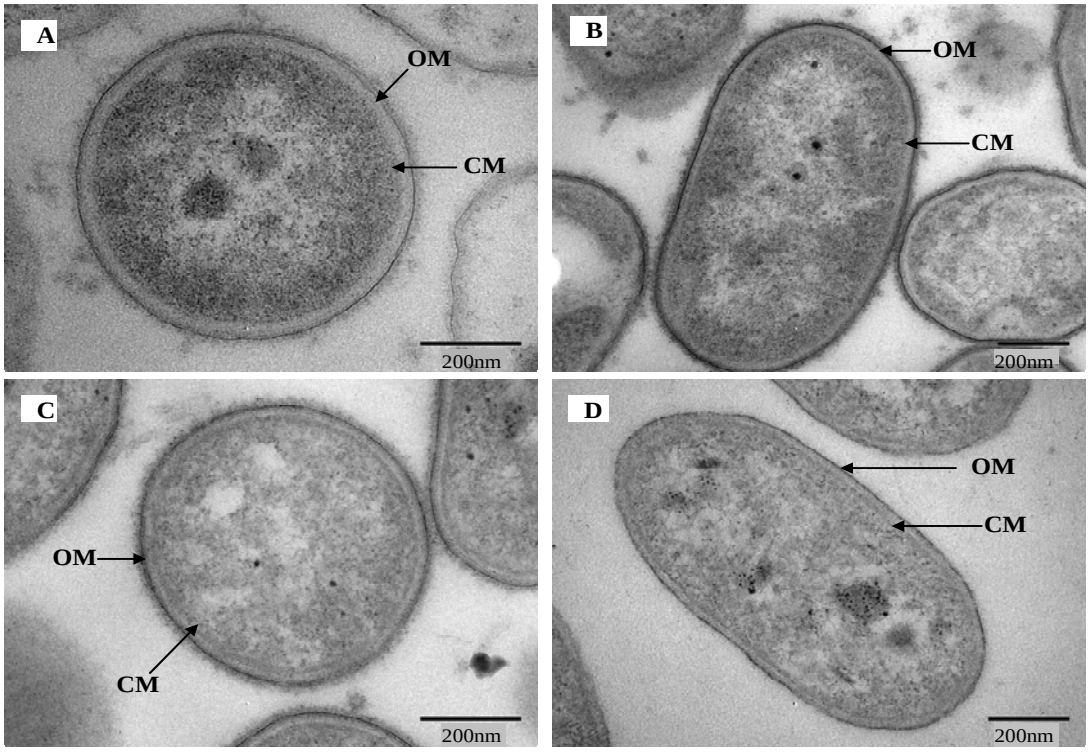


Figure 5-5. *Psedomonas aeruginosa* TEM pictures- A and B are control samples. C and D are triethylsilanol treated samples shows relatively discontinuous OM (outer membrane) and CM (cytoplasmic membrane).

5.3.2 A Study of Membrane Damage of Bacteria by Fluorescent Dye

Membrane damage due to the silanol treatment was monitored by using fluorescent dye through fluorescence microscopy and fluorescence spectroscopy measurement. Four bacterial suspensions without the silanol treatment were tested as control and compared with bacteria treated by the silanols. As expected, the silanol treated bacteria exhibited a reduced green fluorescence light and an increased red fluorescence light. Penetration of the red dye into the bacteria with damaged membrane led to a reduction of the green light. As a result, the silanol treated bacteria showed a double staining fluorescence light with a strong red and a weak green light under fluorescence microscopy as displayed in Figures 5-6 to 5-9. In comparison, bacteria without the silanols treatment showed mostly green light for the Gram-negative bacteria as seen in Figures 5-6 and 5-7, while a strong green light and a weak red light was detected for the Gram-positive bacteria as seen in Figures 5-8 and 5-9. Three fluorescence microscopy pictures for each bacterium are displayed. A is a combined picture of B and C, B is only for green light and C is only for red light. It should be mentioned that bacteria with the red color (Figure C) and the green color (Figure B) are exactly the same bacteria, indicating the double staining on the bacteria. Laflamme et al. (2004) characterized bacterial viability as a function of membrane integrity using fluorescent dye, SYTO 9 and Propidium iodide. In spores with compromised membrane, double staining was detected, and showed that the red light was brighter than the green light.

Fluorescence spectroscopy provides quantitative results, suggesting a reasonable explanation regarding the fluorescence microscopy results. Different concentrations of bacterial suspensions were prepared by diluting from 100% to 90%, 50%, 10%, and 1% as controls. Their fluorescence emissions of each concentration were measured by the

spectrophotometer at a wavelength of excitation of 470nm and an emission of 500-700nm. A relative intensity of the fluorescence emissions at the range between 500 and 550 nm in wavelength where the green light is detected was differentiated depending on the concentration of the bacteria as seen in Figures 5-10. Fluorescence emission corresponding to 10% bacterial concentration represents a 1 log-reduction and 1% is for a 2 log-reduction of viable bacteria. Fluorescence emissions of bacterial suspensions treated by the silanols were compared with the controls. A significant reduction of fluorescence emission at the green light emission wavelength of 500-550nm was observed for the treated bacteria when compared to the 100% control sample as shown in Figures 5-10. The red dye penetrated into the bacteria with compromised membrane, reducing the green light. An increase of red light emission at 620-650nm wavelength was displayed for the treated bacteria. The extent of increase of red light was relatively small except for *Escherichia coli* with triethylsilanol treated shown in Figures 5-10(A).

The fluorescence spectroscopy results showed only a 1 or 2-log reduction of viable bacteria for the silanol treated samples even though the plate count method confirmed a 7-log-reduction. This result is consistent with the fluorescence microscopy result showing double staining for treated bacterial suspension. In other words, the fluorescence emission did not show a zero emission of green light or indicate a 7-log-reduction because the green dye is present with the red dye as double staining for the treated bacteria. Membrane damage of the bacteria by the silanol treatment was investigated by fluorescent dye. The reduction of the green fluorescence light as well as the increase of the red fluorescence light was observed as an indication of a loss of viability of the silanol treated bacteria due to their membrane damage.

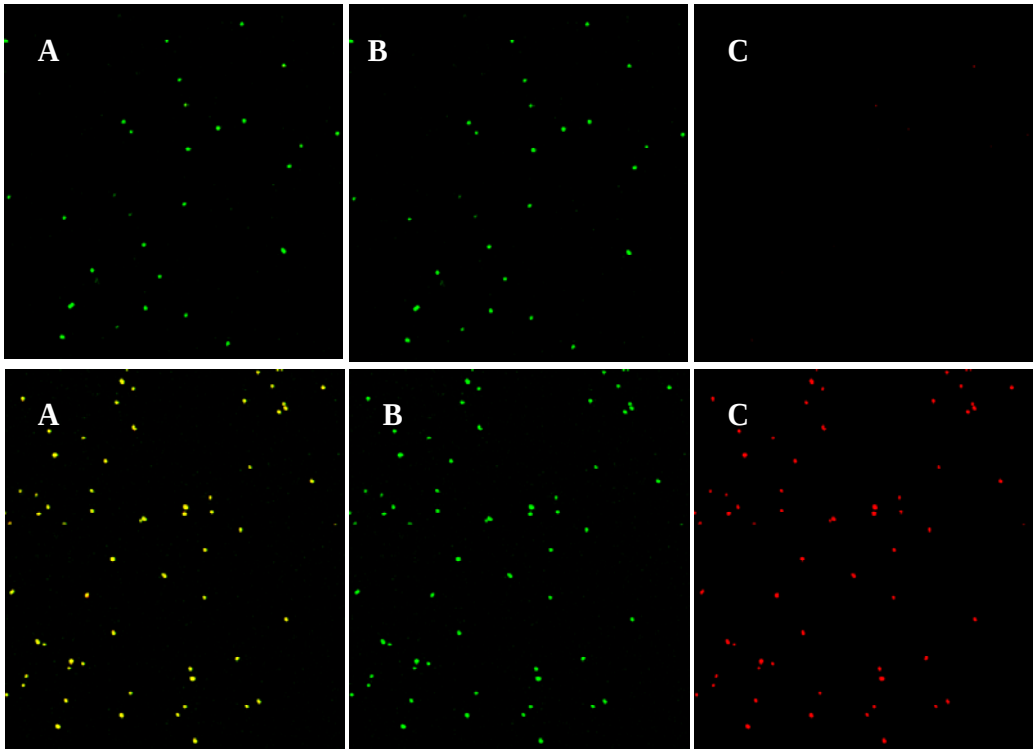


Figure 5-6. Fluoresecent microscopy pictures of *Escherichia coli*, control (top figures) and triethylsilanol treated (bottom figures). A is combined picture of B and C, B is only for green light, and C is only for red light.

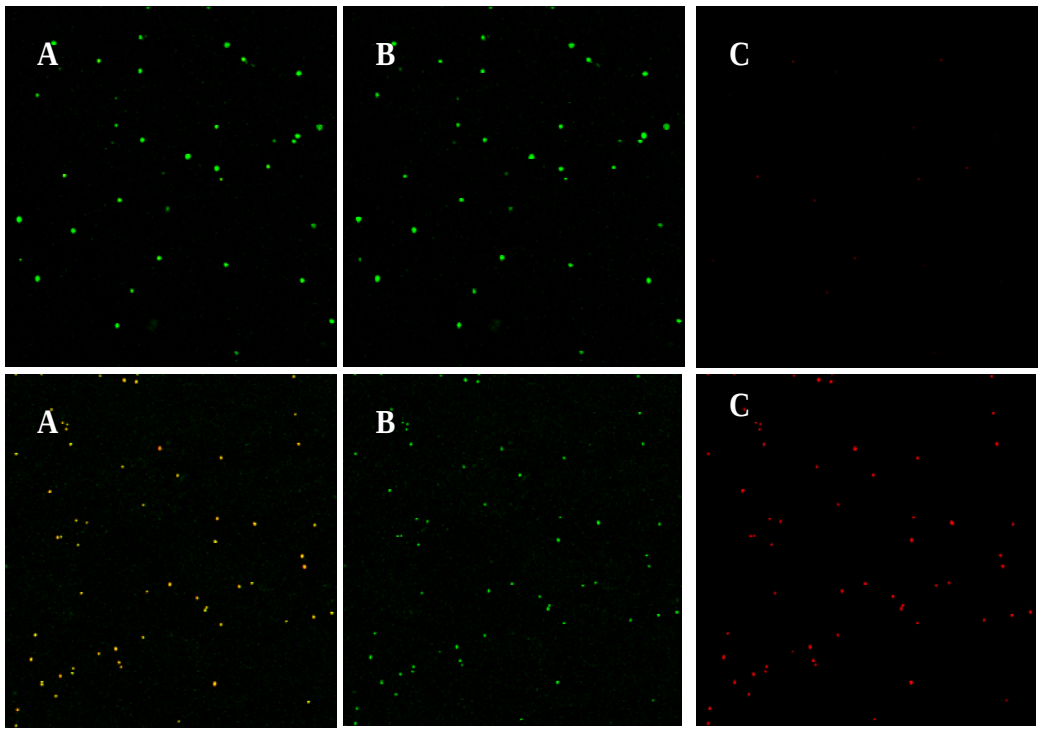


Figure 5-7. Fluoresecent microscopy pictures of *Psedomonas aeruginosa*, control (top figures) and triethylsilanol treated (bottom figures). A is combined picture of B and C, B is only for green light, and C is only for red light.

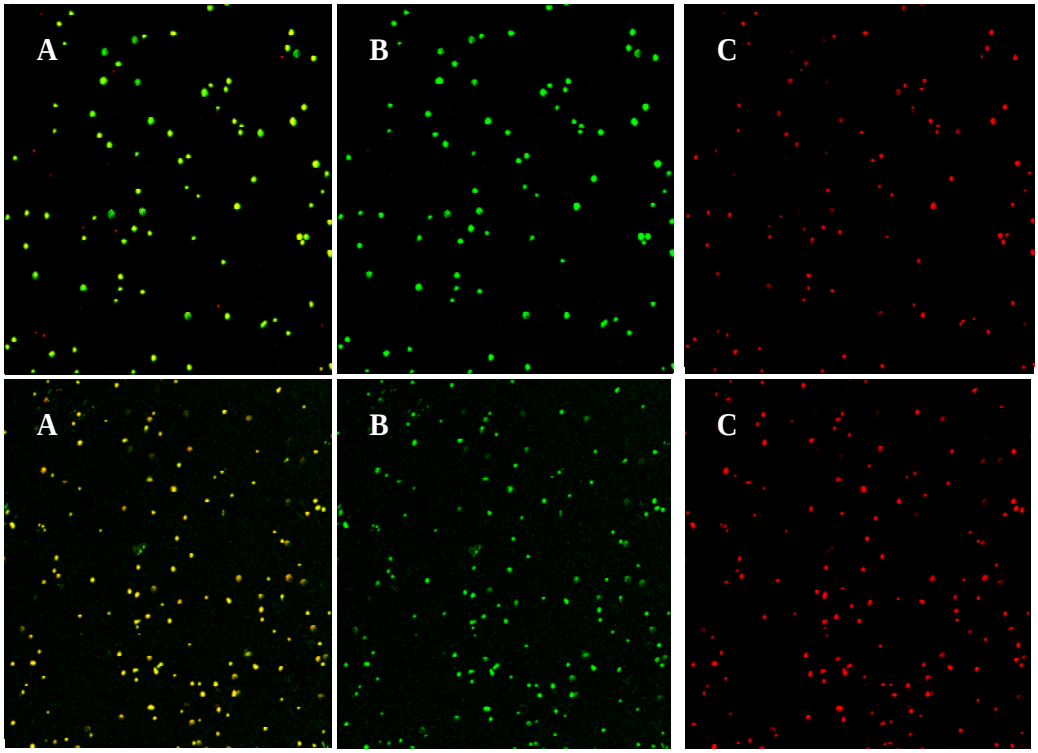


Figure 5-8. Fluoresecent microscopy pictures of *Staphylococcus aureus*, control (top figures) and triethylsilanol treated (bottom figures). A is combined picture of B and C, B is only for green light, and C is only for red light.

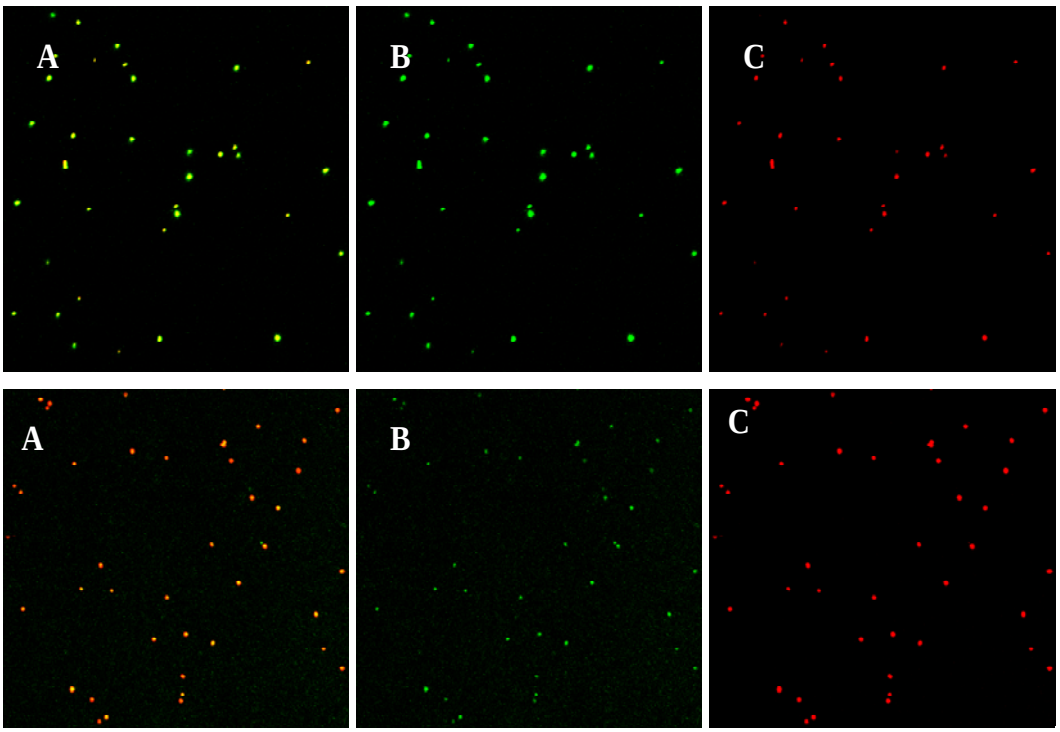
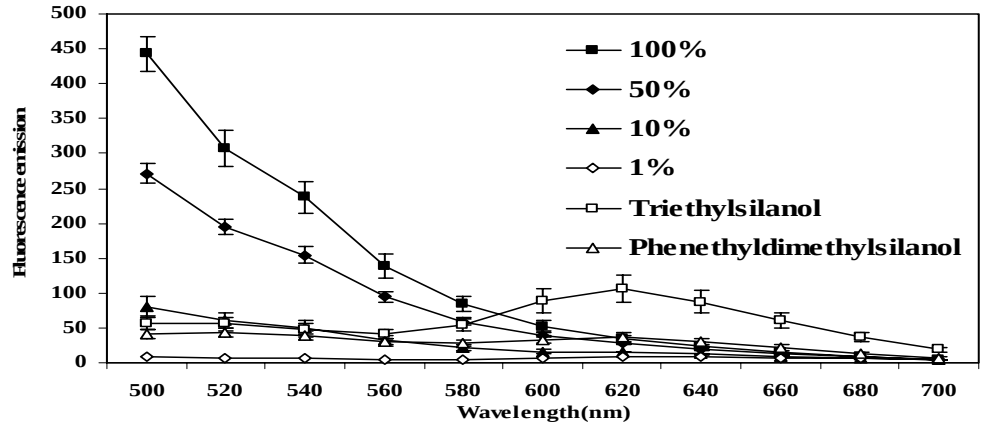
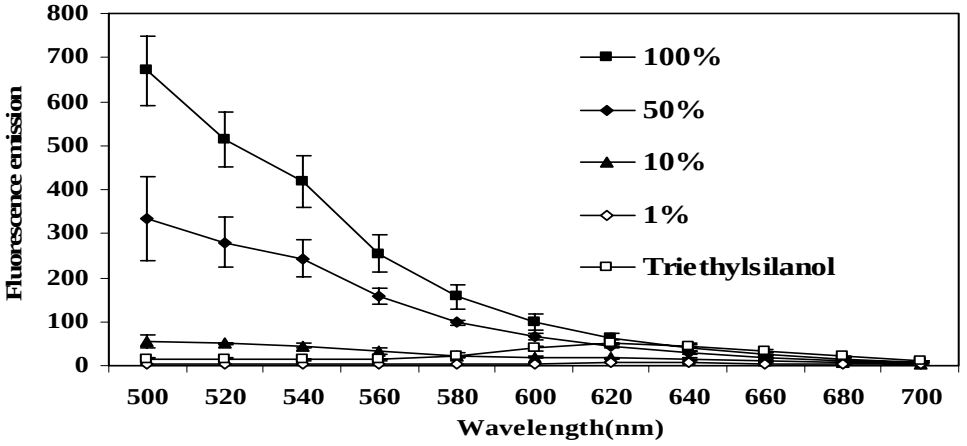


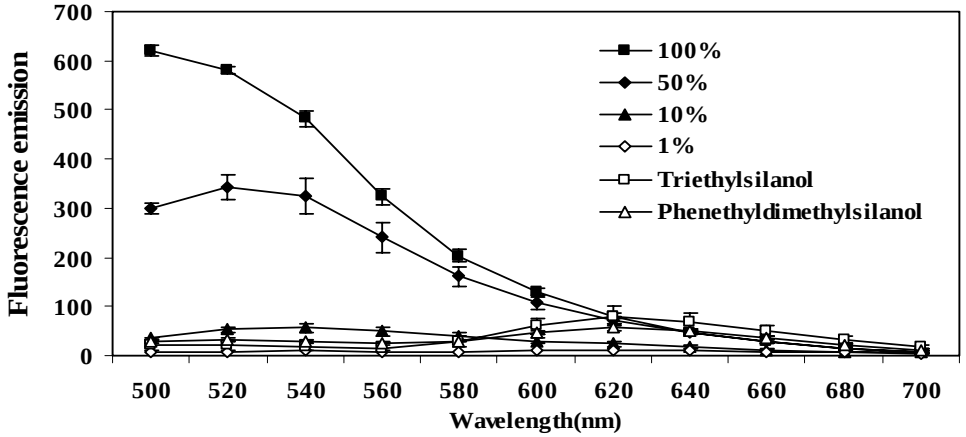
Figure 5-9. Fluoresecent microscopy pictures of *Enterococcus faecalis*, control (top figures) and triethylsilanol treated (bottom figures). A is combined picture of B and C, B is only for green light, and C is only for red light.



(A)



(B)



(C)

Figure 5-10. Fluorescence spectroscopy data for the stained bacteria at emission wavelength 500-700nm with 470nm excitation wavelength. Control samples at concentration of 100%, 50%, 10%, 1% of bacterial suspension and triethylsilanol and phenethylsilanol treated bacterial samples. (A) is *Escherichia coli*, (B) is *Psedomonas aeruginosa*, (C) is *Enterococcus faecalis*, and (D) is *Staphylococcus aureus*.

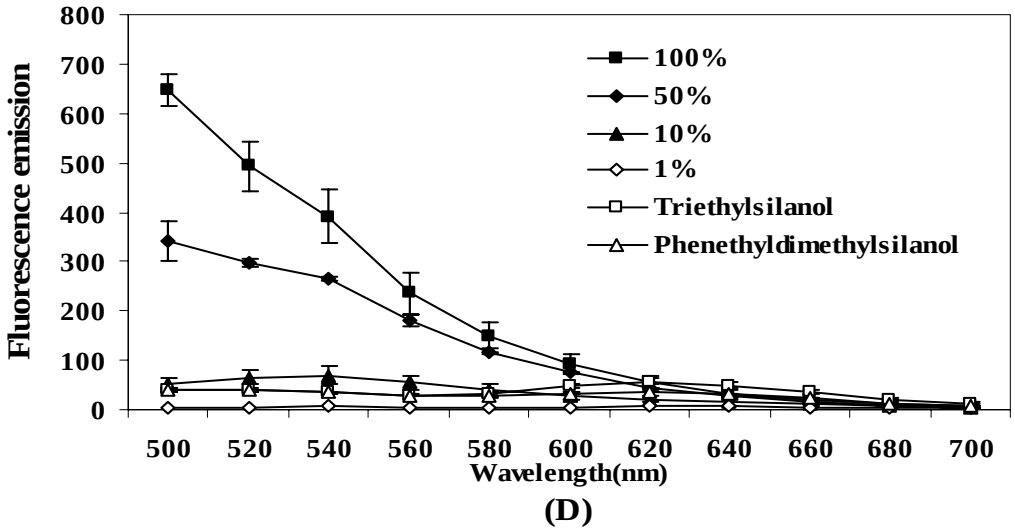


Figure 5-10. Continued

5.4 Conclusions

The mechanism of action of the silanols upon the cell membrane of the bacteria was investigated. A change in the membrane after the silanol treatment was detected by transmission electron microscope, showing a detachment of the plasma membrane from the outer membrane for *Escherichia coli*. In the case of the Gram-positive bacteria a disorganized cytoplasmic membrane was observed upon the silanol treatment when compared to the smooth and intact cell membrane of the untreated bacteria. The fluorescence method allowed to monitor bacteria viability based on the membrane damage because the red dye only penetrates into the bacteria with membrane damage, subsequently reducing the green fluorescence. A significant reduction of the green light, was demonstrated by fluorescent microscopy and spectrophotometer, suggesting that the bacteria treated by the silanol lost their viability due to their membrane disruption.

CHAPTER 6
ANTIMICROBIAL ACTIVITY OF SILANOL TREATED POLYDIMETHYLSILOXANE ELASTOMER (PDMSE)

6.1 Introduction

The use of biocides in plastics or polymer is to protect the material from degradation by microbes and to provide an external antimicrobial hygienic surface. Biocides can be selected based on biocidal performance, in-process stability, and leachability. Antimicrobial plastics have been used as food packaging materials, cutting boards, garbage bags, carpets, surgical gauze and bathroom fixtures, etc.(Mauriello et al., 2005; Vartiainen et al., 2003). Antimicrobials can be added into the plastics during the extrusion process or into the surface coatings as bound antimicrobials. Surface bound antimicrobial activity of an organosilicon quaternary ammonium chloride has been studied extensively (Gottenbos et al., 2002, Isquith et al., 1972; Murray et al., 1988; Walters et al., 1973). Various substrates, such as siliceous surfaces, natural fibers, man-made fibers, metals, and wood, treated by the organosilicon quaternary ammonium chloride have demonstrated antimicrobial activity (Isquith et al., 1972). Quaternary ammonium silane coated silicone rubber showed antimicrobial properties against adhering bacteria such as *Staphylococcus aureus* and *Escherichia coli*, both *in vitro* and *in vivo* (Gottenbos et al., 2002). Bound antimicrobial agents can be used to manufacture sterile bandages, clothing, and gloves as well. In the case of free bound antimicrobials in plastics, mixtures of phenolic antimicrobials such as triclosan and inorganic antimicrobials like silver compounds has been designed for plastic films and fibers by

CIBA specialty chemicals corporation, demonstrated a long-term antimicrobial activity (Herbst and Stadler, 2003). A biocidal plastic or polymer should meet several requirements. It should have a high efficiency with a broad spectrum of microbes, it should maintain its activity throughout its life-time, it should be nontoxic to the environment, and it should be affordable. One of the biggest challenges of free bound antimicrobial in polymers is to control the release rate of the antimicrobials. A controlled release of the antimicrobial is highly recommended not only for the initial inhibition of undesirable microbial growths, but also for maintaining the activity during its life-time.

There is also an increasing interest in developing polymers that inhibit the bio-fouling that occurs in underwater construction and ship hulls. The use of toxic compounds, in particular tributyltin derivatives, dispersed in a polymer matrix or attached to it by chemical bond is now banned for marine applications (Champ, 2000). Research for alternative solutions has been mainly focused on development of an efficient and environmentally friendly product such as silicones. Silicones exhibit excellent marine fouling release properties compared to other materials (Stein et al., 2003; Truby et al., 2000; Wood et al., 2000). In particular, a variety of studies to determine the effects of oil incorporation, surface roughness and topography of silicone have been carried out by Dr. Brennan's group at the University of Florida (Hoipkemeier-Wilson et al., 2004). Incorporating biocides or antifouling agents into silicone coatings was also investigated to achieve a reduction of bacterial attachment and formation of biofilm (Barrios et al., 2005; Haque et al., 2005). Haque et al. (2005) reported that sodium benzoate dispersed in silicone coating showed a reduction of 41-52% in biofilm formation when compared to the control. Barrios et al. (2005) tested Zosteric acid, a natural product present in

eelgrass, for preventing the attachment of bacteria and demonstrated that the bacterial coverage was reduced by 96% for *Pseudomonas putida* at 50ppm of Zosteric acid. At the University of Florida, several silanols, trimethylsilanol, trisiloxanol, and tetrasiloxanol, as antifouling agents have been evaluated by blending into polydimethylsiloxane elastomer (PDMSe). The *Ulva* (syn. Enteromorpha) spore settlement test was performed by Callow's Bioadhesion & Biofouling research group from School of Bioscience in the University of Birmingham, UK. Callow, in a private communication, reported that an initial spore settlement on PDMSe slides containing trimethylsilanol was 20-30% lower than the standard PDMSe. However, approximately no release of the spores was found from the sample with added trimethylsilanol in comparison to approximately 40% removal of settled spores in the flow channel from the standard PDMSe. Callow recommended that further evaluations are required to determine whether the lower number of the spore cells settled on the polymer containing trimethylsilanol was a consequence of loss of vitality of a portion of the swimming spores. Callow also observed that the viability of the swimming spores was compromised against the PDMSe containing trisiloxanol and tetrasiloxanol respectively. This preliminary study suggested that silanols released from the polymer surface could have a strong antimicrobial activity. In this chapter, the antimicrobial activity of the PDMSe containing triethylsilanol or phenethyldimethylsilanol against *Escherichia coli* is evaluated.

6.2 Experiment

6.2.1 Materials

Polydimethylsiloxane elastomer (PDMSe) containing a silanol was prepared. T2 silastic resin, vinyl group ending silicone resin and the T2 silastic curing agent, Pt containing SiH terminated silicone curing agent, were obtained from Essex-Brownell.

Triethylsilanol obtained from Gelest Inc. and phenethyldimethylsilanol synthesized was added into the silicone resin and the curing agents to form polydimethylsiloxane elastomer (PDMSe). Trisiloxanol and tetrasiloxanol were obtained as a gift from Clariant. The purity of the trisiloxanol is 95±2% and 96±2% for the tetrasiloxanol, measured by Gas Chromatography by Clariant.

6.2.2 PDMSe Slide Preparation

PDMSe slides were prepared with a mixing ratio of 10 to 1, T2 Silastic resin and T2 Silastic curing agent respectively. The concentration of triethylsilanol and phenethyldimethylsilanol in PDMSe slides was 4 wt% and 2 wt% respectively. The procedure for preparing the slides is as follows. Several spacers were placed on a large clean glass plate treated by hexamethyldisilazane. A mixture of the silicone resin, the curing agent, and the silanol was prepared at the given ratio, 10:1:0.22 for phenethyldimethylsilanol mixture and 10:1:0.45 for triethylsilanol mixture. The mixture was placed into a vacuum chamber for degassing for 2-3 minutes. The mixture was evenly poured on a glass plate followed by removal of bubbles using a needle to pop the bubbles. Another large clean glass plate also treated by hexamethyldisilazane was slowly placed on top of the sample. The slides were cured at 50 ºC for 5 hours. A control sample, consisting of Silastic T2 base and curing agent only, was prepared as well. The dimension of the PDMSe slide is 76mm by 25mm with film thicknesses of approximately 1mm. All the PDMSe slides were quickly rinsed by ethanol using a Kim-wipe to remove residual leachate on the sample surface.

6.2.3 Aqueous Suspension Antimicrobial Experiment

125ml flask contained a 76mm by 25mm silanol treated polydimethylsiloxane elastomer slide with approximately 0.12 g of triethylsilanol and 0.06 g of

phenethyldimethylsilanol in 30 ml of bacterial solution with a concentration of 2-6×10^7 cfu/ml. The solution was constantly swirled by a shaker at room temperature. Samples taken from the solution was evaluated by the plate count method (Collines, 1995; Franson, 1985). The experiment was performed as a function of exposure time as well as aging of silanol treated PDMSe.

6.2.4 Zone of Inhibition Test

A quantity of 0.1ml of bacterial suspension containing about 2-6×10^7 cfu of *Escherichia coli* was spreaded on agar plates. The silanol treated PDMSe slide sample, approximately 38mm×25mm, was placed in contact with the agar surface, and the plate was incubated at 37 ºC for 24 hours. After the incubation, the antimicrobial activity was observed by the absence of growth of *Escherichia coli* in the part of the plate in contact with the PDMSe sample (Mauriello et al., 2004).

6.3 Results and Discussion

6.3.1 Antimicrobial Activity of Silanol Treated PDMSe in Aqueous.

The PDMSe slides were prepared by incorporating silanols into the polymer system as antimicrobial polymer. Inactivation of viable bacteria by free bound silanol in the PDMSe was observed as a function of exposure time and aging of the silanol treated PDMSe. Silanol concentration of the 76mm by 25mm silanol treated PDMSe slide in 30 ml of the bacterial solution should be approximately 0.4 wt% for triethylsilanol and 0.2 wt% of phenethyldimethylsilanol if there was no loss of the silanol during the PDMSe preparation. However, the actual concentration might be lower due to a loss of the silanol during the degassing and the curing process. For the degassing, weight loss was relatively insignificant. The control lost 0.04 wt%, the triethylsilanol treated sample lost 0.07 wt%, and the phenethyldimethylsilanol treated one lost 0.05 wt%. After the samples were cured

the silanol treated PDMSe continuously lost their weight in the air. While the treated PDMSe slide was kept in the hood for 3 days a weight change of the sample was measured. Triethylsilanol treated PDMSe slide lost 0.95±0.09 wt% and the phenethyldimethylsilanol sample lost 0.4±0.03 wt% within 24 hours while untreated PDMSe showed only 0.1 wt% loss. Further weight loss within the second and third day was no more than 0.1 wt%. A change in the antimicrobial activity of the aged sample was observed because the free bound silanol in the PDMSe was leaching out into the air within the container where the samples were kept. There was a log reduction difference in the number of viable bacteria between the treated PDMSe and the untreated PDMSe. It should be pointed out that the minimum lethal concentration of triethylsilanol was 0.2 wt% and 0.1wt% for phenethyldimethylsilanol. Hence, the treated PDMSe slide with the size of 76mm by 25mm containing 0.4 wt% of triethylsilanol or 0.2 wt% of phenethyldimethylsilanol should be capable of demonstrating a 7-log reduction when the free bound silanol releases out of the PDMSe and contact the bacteria. The log reduction of *Escherichia coli* in aqueous experiment is summarized in Table 6-1 as a function of contact time and age of sample. Figure 6-1 for triethylsilanol treated PDMSe and Figure 6-2 for phenethyldimethylsilanol treated PDMSe clearly showed an increase of log reduction as a function of contact time. An effect of sample age was significant for triethylsilanol, showing a reduction of antimicrobial activity as the sample ages. In comparison, the aging effect for phenethyldimethylsilanol treated PDMSe was insignificant for the log reduction rate for the different aged samples as shown in Figure 6-2. It is suspected that the release rate of triethylsilanol from the PDMSe was faster than that of phenethyldimethylsilanol. As a result of the difference in release rate, a 1-log

reduction, meaning 90% kill, of *Escherichia coli* took place within 6 hours for triethylsilanol except for the 3 day aged sample, whereas a 1-log reduction took more than 12 hours for the phenethyldimethylsilanol treated PDMSe. In the case of the triethylsilanol treated PDMSe, a 7-log reduction required less than 24 hours while the phenethyldimethylsilanol treated PDMSe showed only a 4-log reduction after 48 hours of contact time. We hypothesized that the release rate is related to the octanol/water partition coefficient of silanols since silanol resides in the PDMSe which is relatively hydrophobic, similar to the octanol. The partition coefficient of triethylsilanol is 2.62, lower than that of phenethyldimethylsilanol which is 3.34. Hence, a release rate of triethylsilanol should be much faster than the release rate of phenethyldimethylsilanol from the PDMSe. A clear trend of log reduction as a function of time was observed however, when the log reduction value is small, a relatively large error is obtained when calculating the standard deviation of 3 data points, as presented in Table 6.1.

Table 6-1. Measure of log reduction of *Escherichia coli* by silanol treated PDMSe in an aqueous solution.

	Triethylsilanol (4 wt%)				Phenethyldimethylsilanol (2 wt%)		
Time	1 day aging	2 day aging	3 day aging	Time	1 day aging	2 day aging	3 day aging
2hr	0.03±0.02	0.08±0.06	0.03±0.02	4hr	0.04±0.04	0.05±0.05	0.08±0.07
4hr	0.99±0.88	0.37±0.15	0.10±0.06	8hr	0.17±0.14	0.30±0.23	0.35±0.3
6hr	2.90±1.53	1.05±1.25	0.24±0.19	12hr	0.70±0.46	0.74±0.1	1.06±0.78
8hr	5.61±1.92	2.47±2.44	0.62±0.5	24hr	2.64±1.32	3.62±0.65	2.66±1.77
12hr	6.12±1.58	4.90±2.56	2.26±1.45	36hr	4.11±0.24	4.36± 0.2	3.62±1.35
24hr	7.84±0.04	6.36±1.21	4.34±3.65	48hr	4.31±0.21	4.43±0.22	4.03±0.65

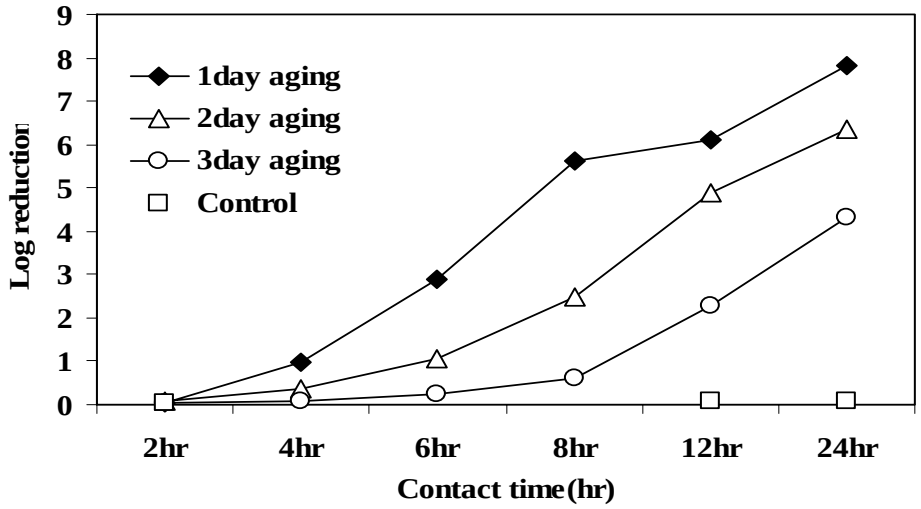


Figure 6-1. Log reduction of *Escherichia coli* upon triethylsilanol(4wt%) treated PDMSe in aqueous solution. Control is untreated PDMSe.

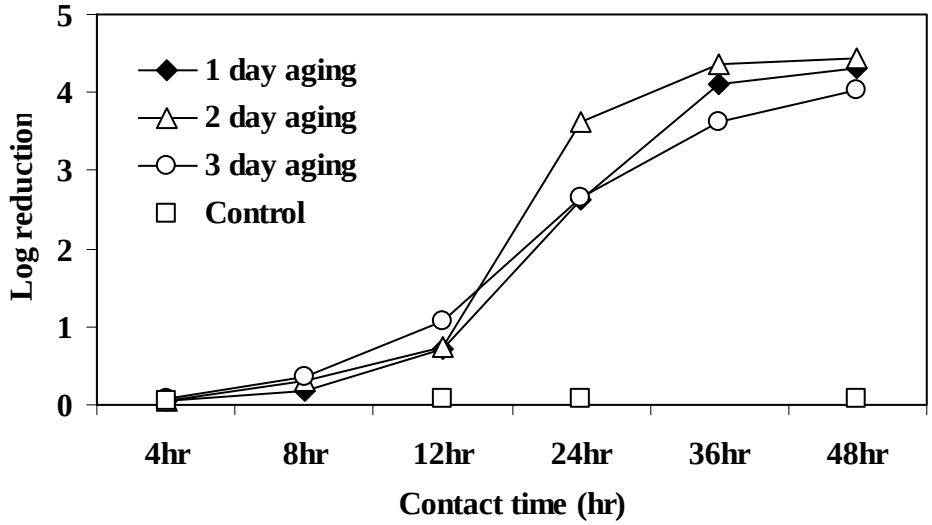


Figure 6-2. Log reduction of *Escherichia coli* upon phenethyldimethylsilanol (2wt%) treated PDMSe in aqueous solution. Control is untreated PDMSe.

6.3.2 Zone of Inhibition by an Silanol Treated PDMSe.

The antimicrobial activity of the silanol treated PDMSe was performed by different methods. One measured the kill rate in an aqueous solution as a function of time, the other one showed the zone of inhibition of bacteria growth. In Figure 6-3, triethylsilanol treated PDMSe clearly showed regions where *Escherichia coli* did not grow or population of the colony was significantly reduced while in the agar plate of untreated

PDMSe *Escherichia coli* grew homogeneously on the surface. The inhibition region for phenethyldimethylsilanol treated PDMSe was limited to an area close to the PDMSe sample unlike the triethylsilanol treated sample as shown in Figure 6-3(C). It is speculated that triethylsilanol leachate from the PDMSe released faster and diffused into the agar nutrient more efficiently than that of phenethyldimethylsilanol. The log reduction experiment also supports the differences observed in the zone of inhibition test because only a 4-log reduction occurred for the phenethyldimethylsilanol treated sample after 48 hours of contact when compared to the 7-log reduction for the triethylsilanol sample after 24 hours of contact.

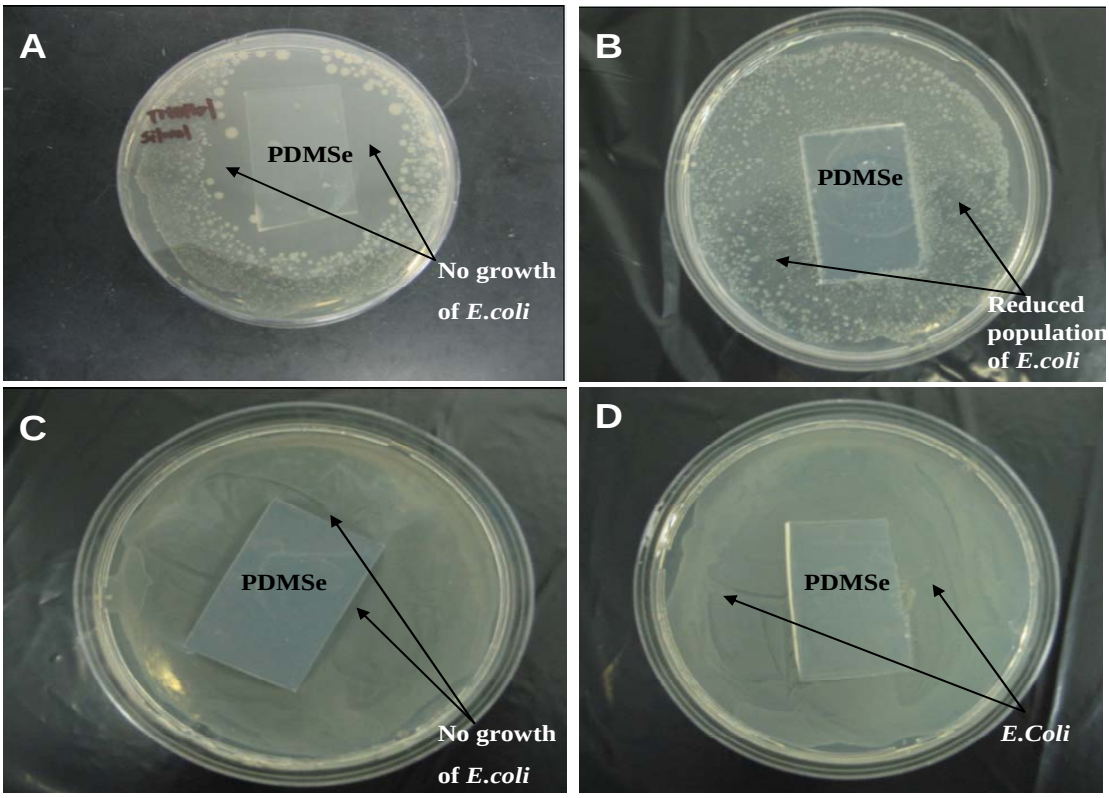


Figure 6-3. Zone of inhibition test results against *Escherichia coli*, A is triethylsianol treated PDMSe, B is the other triethylsilanol treated PDMSe, C is phenethyldimethylsilanol treated PDMSe, D is control (untreated PDMSe).

6.3.3 Antimicrobial Activity of Siloxanols and Trimethylsilanol against the *Ulva* Spore in Comparison with *Escherichia coli* Test.

In the previous experiment with *Ulva* spore, the green algal genus, the PDMSe slide containing 2 wt% of trisiloxanol or tetrasiloxanol demonstrated some level of toxicity associated with the polymer slide. Callow, in a private communication, reported that the poor autofluorescence of spores indicates a loss of chlorophyll and the transmitted light test revealed a high proportion of mis-shapen spores. Callow also observed outstretched flagella indicating that spores had lost motility, and cell debris distributed across the surface. Antimicrobial testing was performed with a neat trisiloxanol and tetrasiloxanol in aqueous solution to measure their bioactivity against *Escherichia coli*. No antimicrobial activity against *Escherichia coli* was observed at a concentration of 2.5% of the siloxanols in aqueous solutions as shown Figure 6-4. A low level of toxicity associated with the PDMSe containing 2 wt% of trimethylsilanol was also reported by Callow. In Figure 6-4, a 7-log reduction of *Escherichia coli* upon a 2.5 wt% of trimethylsilanol treatment was observed when compared to a less than a 1-log reduction for trisiloxanol and tetrasiloxanol respectively. The siloxanols showed a low antimicrobial activity against *Escherichia coli* possibly due to their too high hydrophobicity. The partition coefficient value for trisiloxanol was 3.39 and 3.98 for tetrasiloxanol. Antimicrobial activity of the siloxanols seems to vary with microorganisms. Additional experiments are recommended for the siloxanols against the *Ulva* spore to confirm the toxicity reported by Callow.

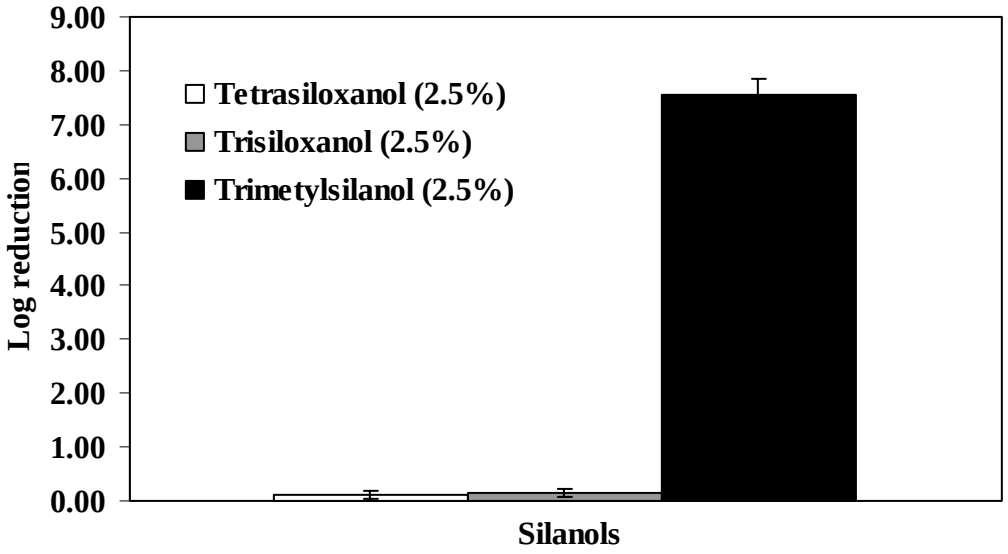


Figure 6-4. Antimicrobial activity of siloxanols and trimethylsilanol against *Escherichia coli* in an aqueous solution at a concentration of 2.5 (%g/g).

6.4 Conclusion

A polydimethyldisiloxane elastomer, Silastic T2, was treated by triethylsilanol and phenethyldimethylsilanol to measure the log reduction of the viable *Escherichia coli* as a function of contact time and aging of the samples. It was revealed that the kill rate was related to the partition coefficient of the silanols, and demonstrated that the triethylsilanol treated PDMSe showed a 7-log reduction within 24 hours of contact while the phenethyldimethylsilanol treated sample showed only a 4-log reduction for 48 hours of contact. The zone of inhibition test was also consistent with the results of the log reduction test, supporting the notion that the triethylsilanol released by the PDMSe was faster than that of phenethyldimethylsilanol. The siloxanol samples were tested against two different microorganisms. No antimicrobial activity was observed against *Escherichia coli* in comparison with some level of toxicity with *Ulva* spore. Further study is recommended to verify the variation of the toxicity.

CHAPTER 7
CONCLUSIONS AND FUTURE WORK

7.1 Conclusions

Antimicrobial activity of silanols, so called silicon alcohols, has recently been discovered (Kim et al., 2006). Silanols antimicrobial activity was at least twice as strong as those of analogous alcohols consistent with toxicity of trimethylsilanol compared with its analogous alcohol, t-butanol, reported earlier by Bennett and Staratt (1973). It was originally hypothesized that silanols were stronger antimicrobials than analogous alcohols due to their high lipophilicity, to the hydrogen bond acidity, and formation of extracoordination. In this study the lipophilicity and the hydrogen bond acidity were found to be primary factors, therefore considering the extracoordination as a possible factor for the enhanced antimicrobial effect of the silanols was eliminated in this study.

In order to investigate the antimicrobial action two approaches were employed. One approach was that two structural properties of the silanols were correlated with the antimicrobial activity of the silanols to investigate how the structural parameters of the antimicrobials affect the degree of inactivation of bacteria, and to understand the kill mechanisms of the bacteria upon silanols treatment. In our study, silanols, alcohols, and phenols were treated as a single class of antimicrobials because their chemical structures are similar, consisting of a hydrophobic organic group and a hydrophilic hydroxyl group. Correlation equations between the structural parameters and the antimicrobial activity were established by using multiple regression analysis method for each bacterium, *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Enterococcus*

faecalis, with silanols, alcohols, and phenols. A linear free-energy relationship was established between the antimicrobial activity and the structural parameters, revealing that the substituents lipophilic nature and the H-bond acidity of silanols, alcohols, and phenols contribute to their antimicrobial activity. In other words, the antimicrobial activity increases as their lipophilicity increases as well as the H-bond acidity increases. However, there was a cut off point where the activity begins to decrease as the lipophilicity continuously increases. The effect of the H-bond acidity to the antimicrobial activity was relatively small compared to the lipophilicity. This study supports that silanols, alcohols, and phenols are part of the same family, thus following the same kill mechanisms of bacteria including membrane damage due to the hydrophobic interaction between the hydrophobic part of the membrane and the organic group of the antimicrobials. The correlation equations corresponding to each bacterium were also similar indicating that a mechanism of bio-response of the four bacteria with the antimicrobials may follow the same antimicrobial action.

The other approach was a more direct method to observe the disruption of bacteria membrane due to the hydrophobic interaction by using transmission electron microscopy (TEM) and fluorescent dye. This hydrophobic interaction can lead to a loss of the function of the membrane, which is a selective permeability barrier, and subsequently cause the death of bacteria. A disruption of the membrane of bacteria after the silanols treatment was detected by TEM, showing as a detachment of the plasma membrane from the outer membrane, as in the case of *Escherichia coli*. For the Gram-positive bacteria, disorganized cytoplasmic membrane was clearly observed for silanols treated bacteria when compared to the smooth and intact cell membrane on untreated bacteria. A

fluorescence method was also used to monitor the viability of bacteria as a function of membrane damage because the red dye only penetrates into bacteria with membrane damage, subsequently reducing the green fluorescence light. A significant reduction of the green light was demonstrated by fluorescent microscopy and spectrophotometer, suggesting that bacteria treated by silanols lost their viability due to their membrane disruption.

Silanols can be found in low molecular silicones such as decamethylcyclopentasiloxane (D5) and in high molecular silicones such as polydimethylsiloxane as a hydrolysis product or from incomplete crosslinking. Organopolysiloxanes are important materials for health and personal care applications. Therefore, discovery of antimicrobial activity of silanols could raise concerns about toxic effect of silanols when silicones are used for health and personal care related products. Toxic effect of the silanols can be concentration dependent and vary with different types of biological systems.

7.2 Future Work

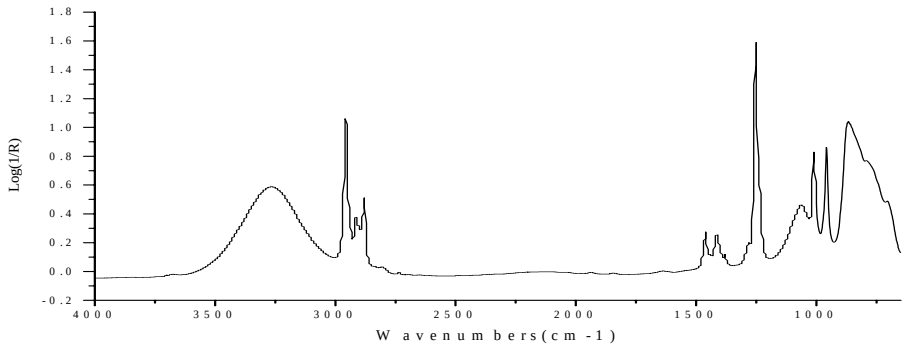
One of the advantages of silanols over alcohols is that silanols can be used as chemically bound antimicrobials. Silanol can readily bind onto substrates containing hydroxyl groups such as textiles, plastics, metals or woods. A study of control release of silanol from the substrates is recommended to inactivate bacteria as well as maintain biocidal property for the life-time of the substrates.

It has been shown that the lipophilicity and the H-bond acidity are the primary factors for the antimicrobial activity of silanols. The antimicrobial activity of silanols decreased when the silanol was too hydrophobic or too hydrophilic. Hence, antimicrobial

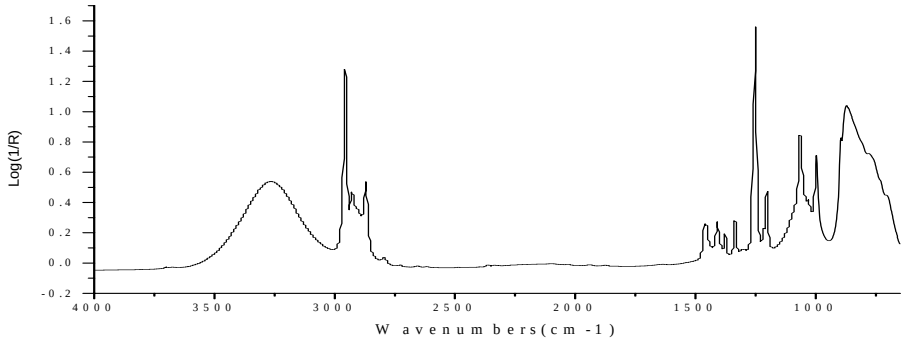
activity of the silanol can be optimized or maximized by engineering chemical structure of silanols and by adjusting the two key parameters.

In this study, the tertiary structure of silanols and alcohols, and mono-substituted phenols were tested to set up the correlation equations, which can be a tool for predicting antimicrobial activity of unknown compounds. Addition of different chemical structure types such as n-primary structure of alcohols or diol structure for the correlation model can improve the predictive ability of the models.

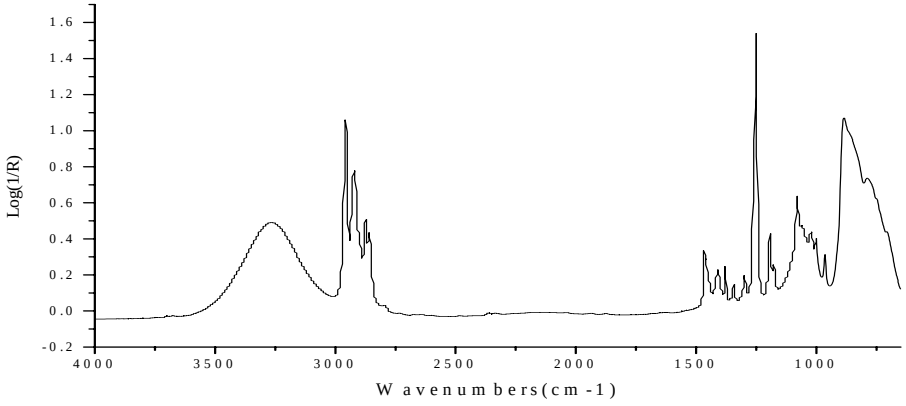
APPENDIX A
FTIR AND NMR DATA



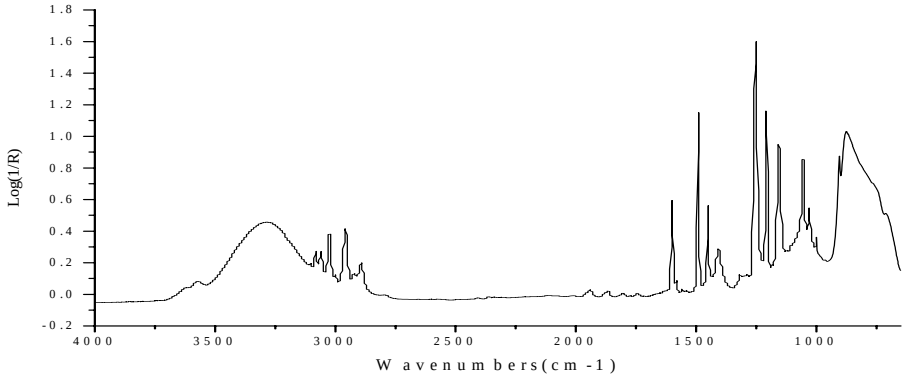
A-1. An infrared spectrum of ethyldimethylsilanol



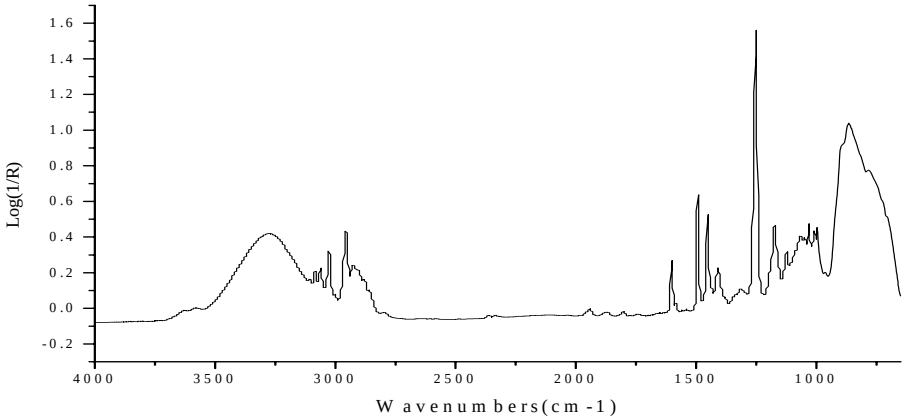
A-2. An infrared spectrum of n-propyldimethylsilanol



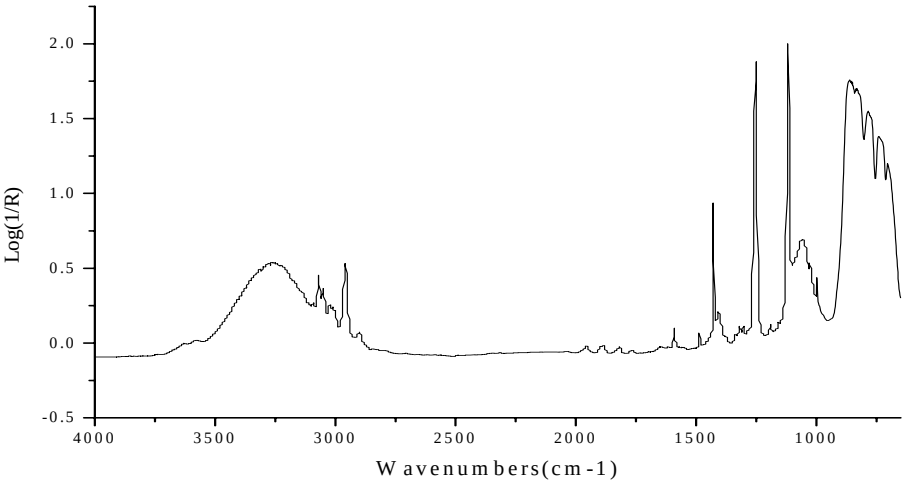
A-3. An infrared spectrum of n-butyldimethylsilanol



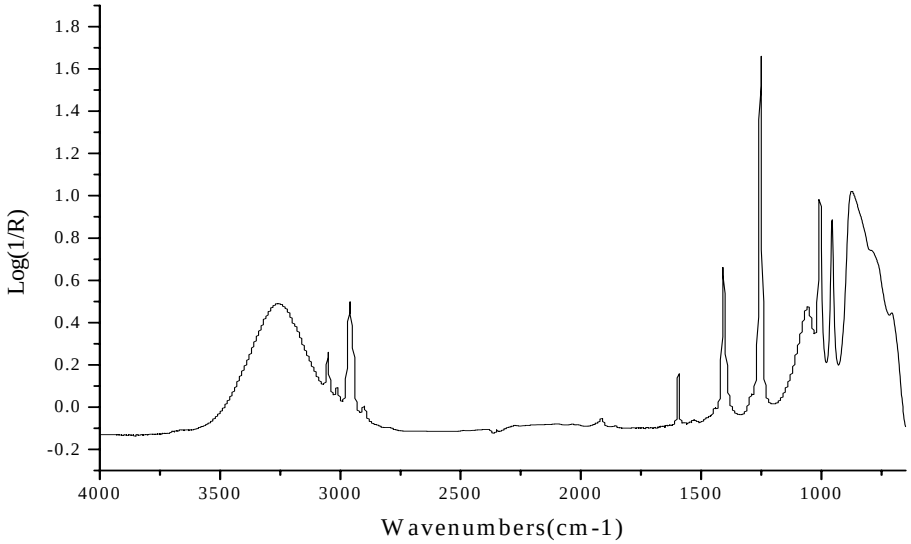
A-4. An infrared spectrum of benzyldimethylsilanol



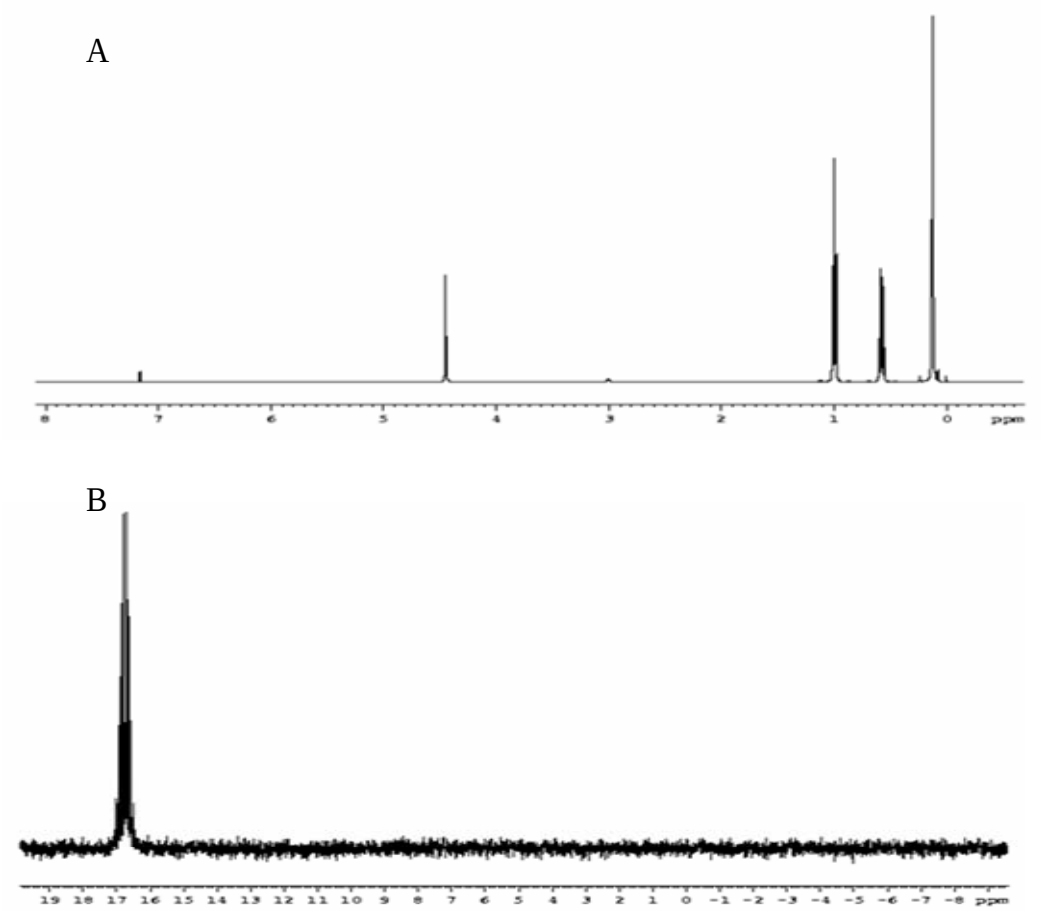
A-5. An infrared spectrum of phenethyldimethylsilanol



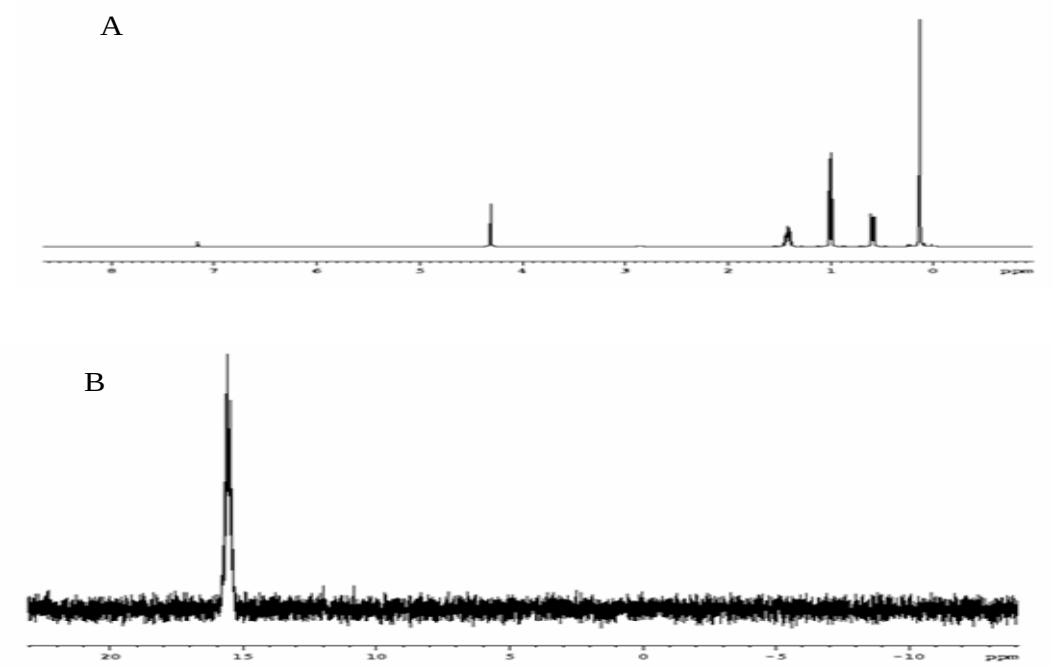
A-6. An infrared spectrum of phenyldimethylsilanol



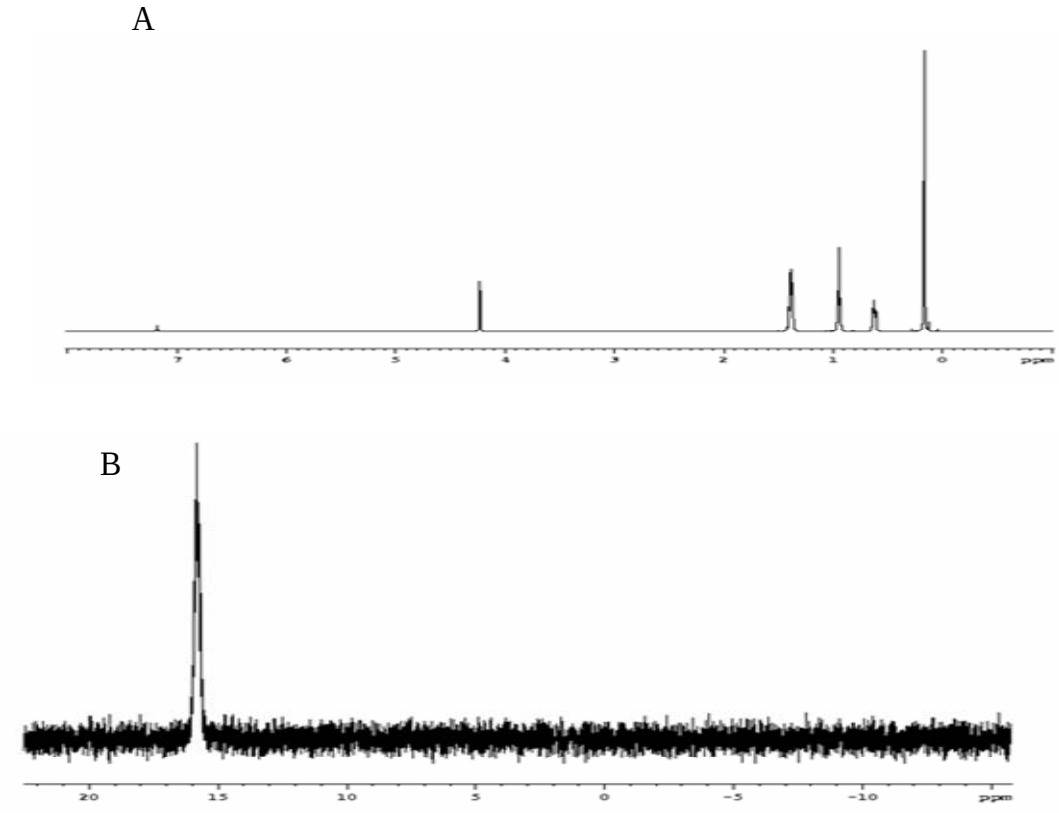
A-7. An infrared spectrum of vinyldimethylsilanol



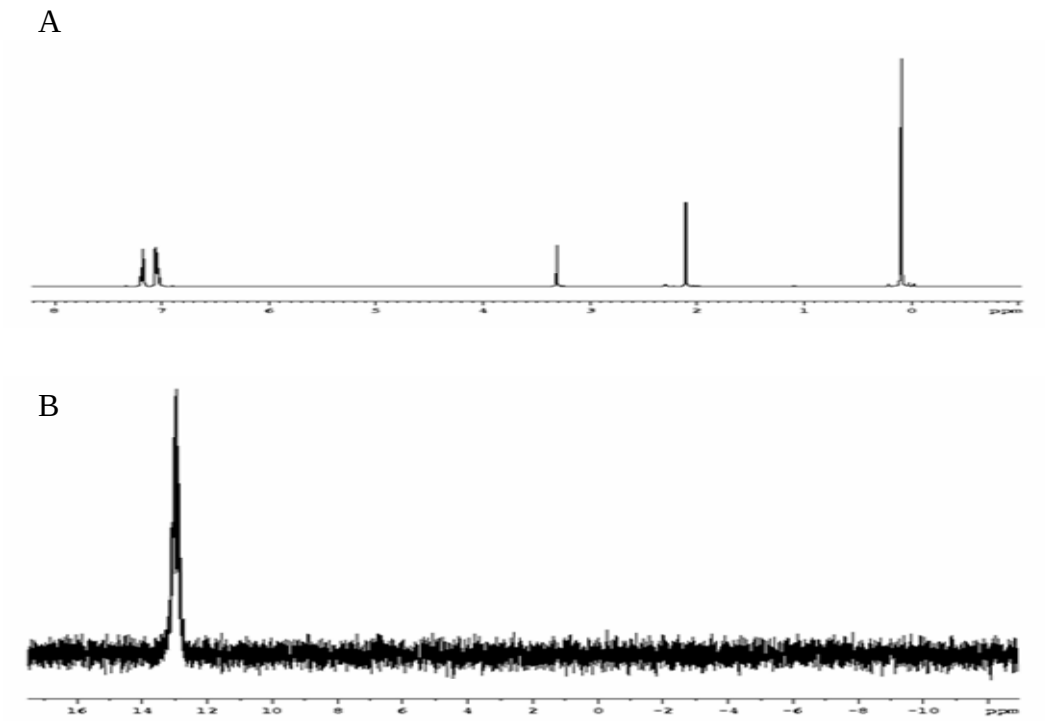
A-8. NMR spectra of ethyldimethylsilanol, A is H-1 NMR, B is Si-29 NMR in Benzene-D_6 solvent.



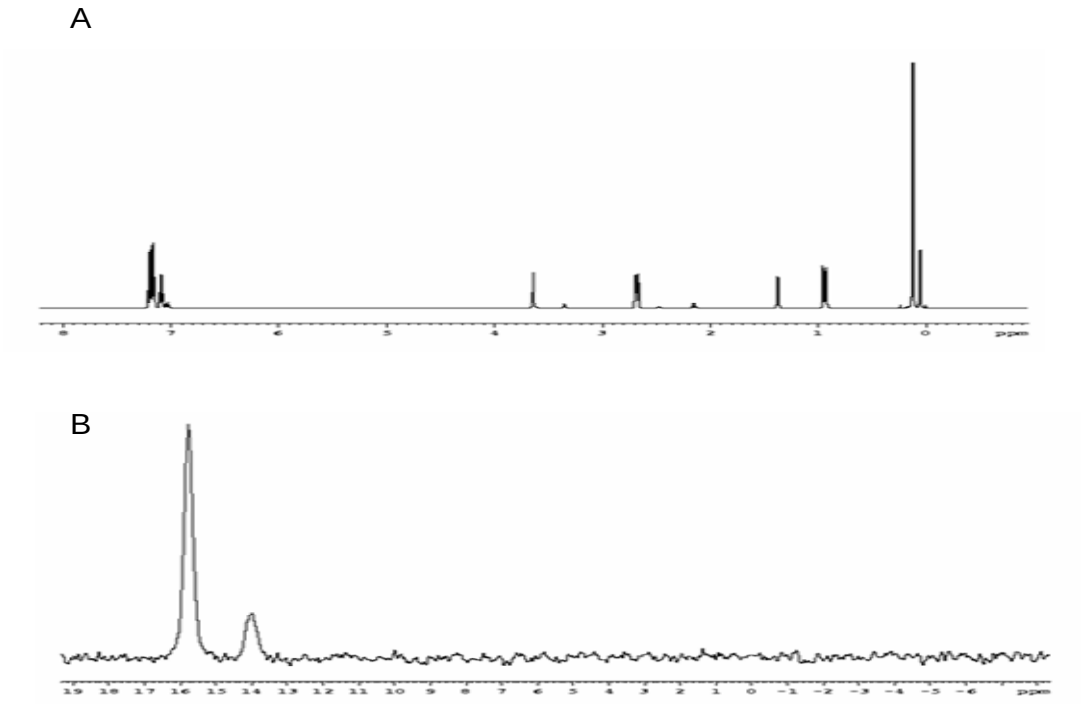
A-9. NMR spectra of n-propyldimethylsilanol, A is H-1 NMR, B is Si-29 NMR in Benzene-D_6 solvent.



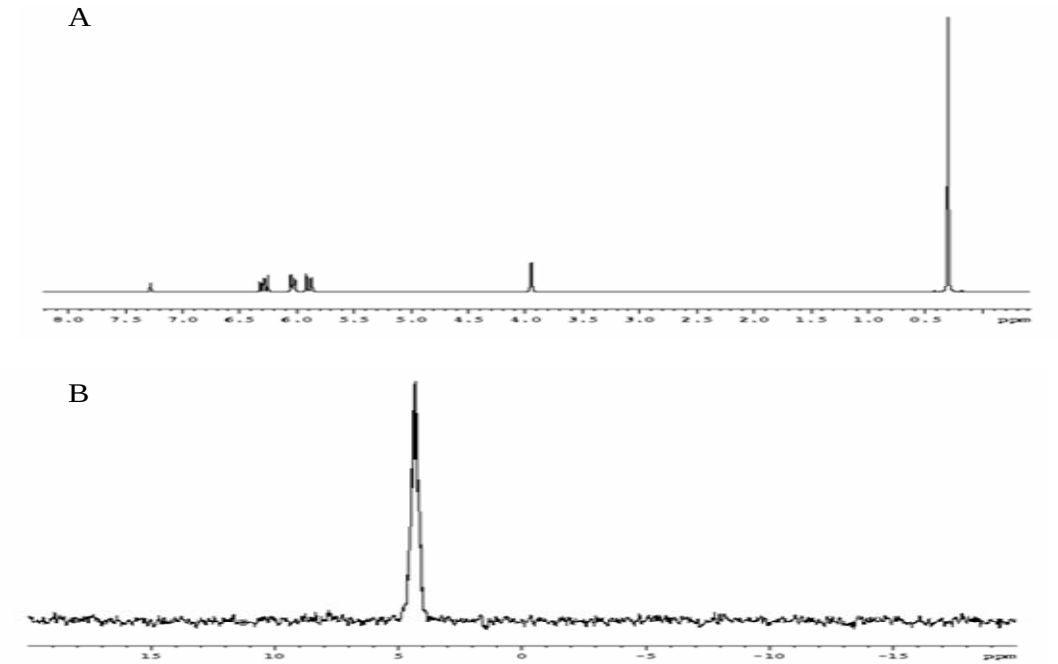
A-10. NMR spectra of n-butyldimethylsilanol, A is H-1 NMR, B is Si-29 NMR in Benzene-D_6 solvent.



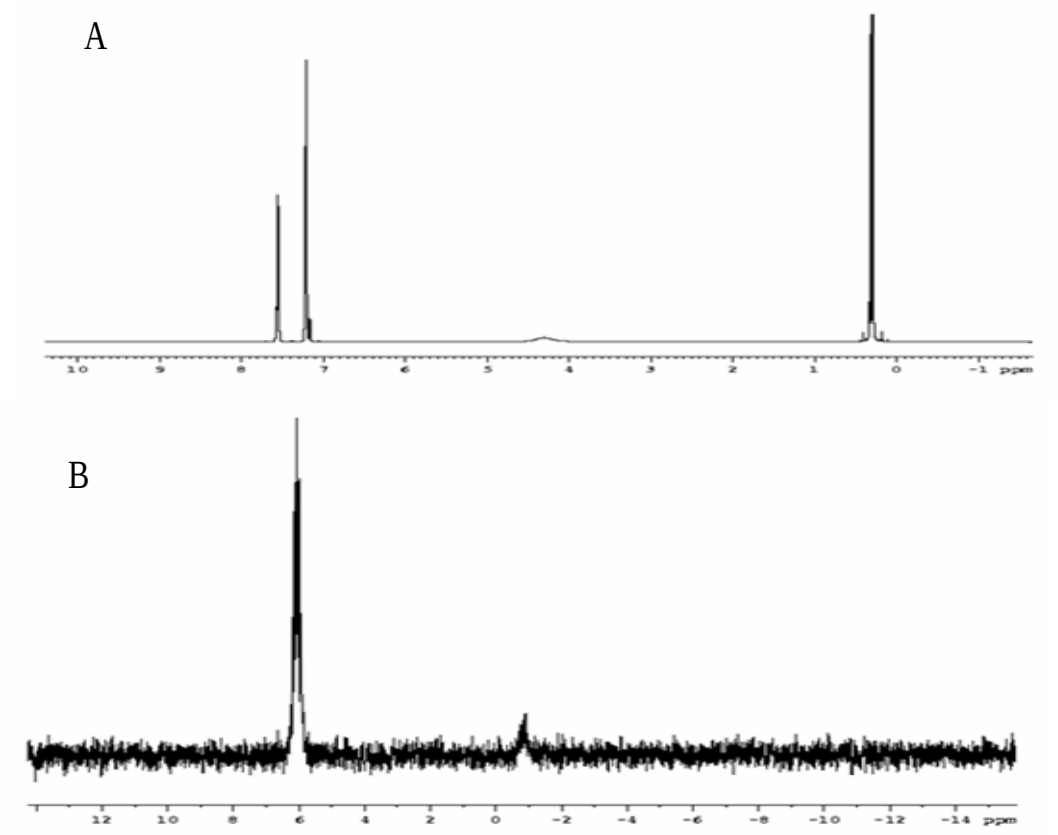
A-11. NMR spectra of bezyldimethylsilanol, A is H-1 NMR, B is Si-29 NMR in Benzene-D_6 solvent.



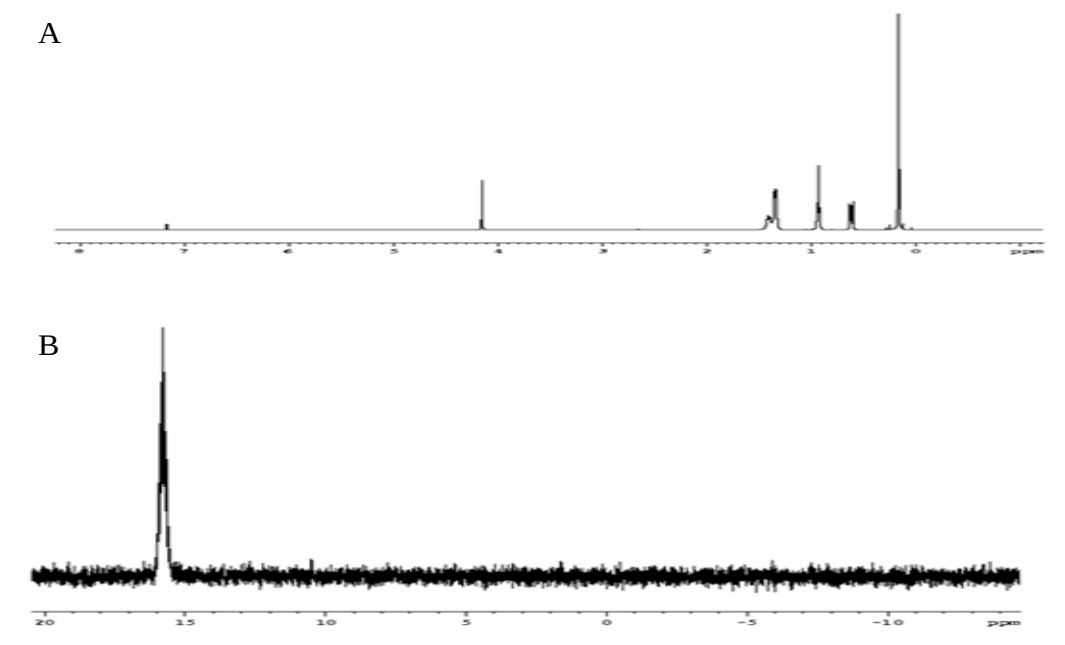
A-12. NMR spectra of phenethyldimethylsilanol, A is H-1 NMR, B is Si-29 NMR in Benzene-D_6 solvent



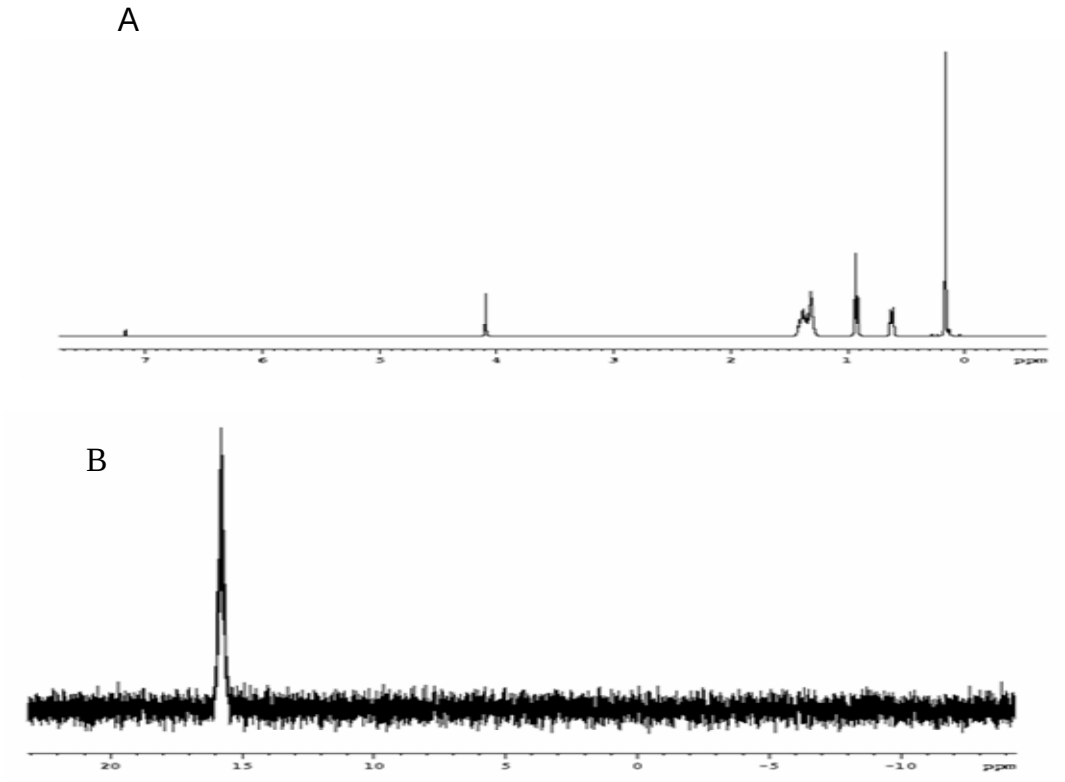
A-13. NMR spectra of vinyldimethylsilanol, A is H-1 NMR, B is Si-29 NMR in Benzene-D_6 solvent



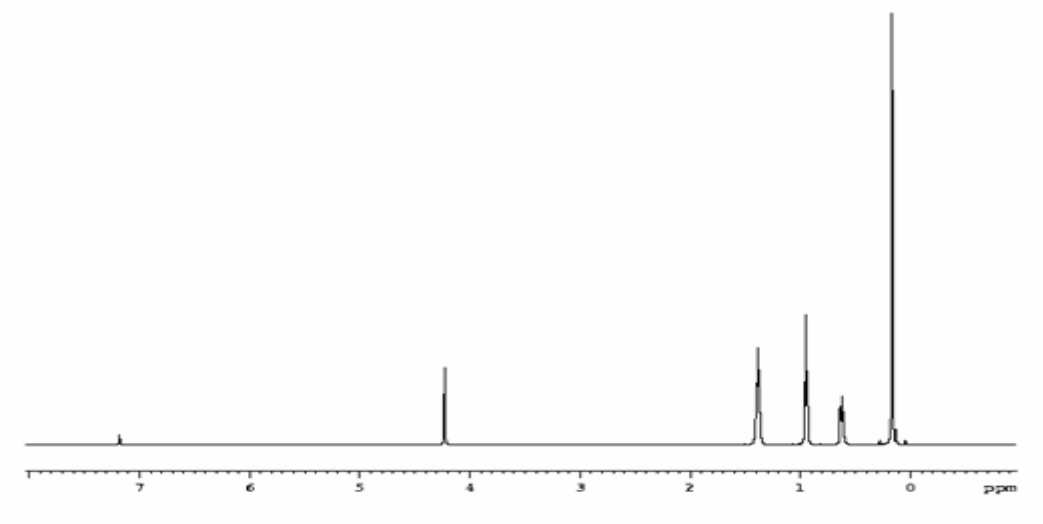
A-14. NMR spectra of phenyldimethylsilanol, A is H-1 NMR, B is Si-29 NMR in Benzene-D_6 solvent.



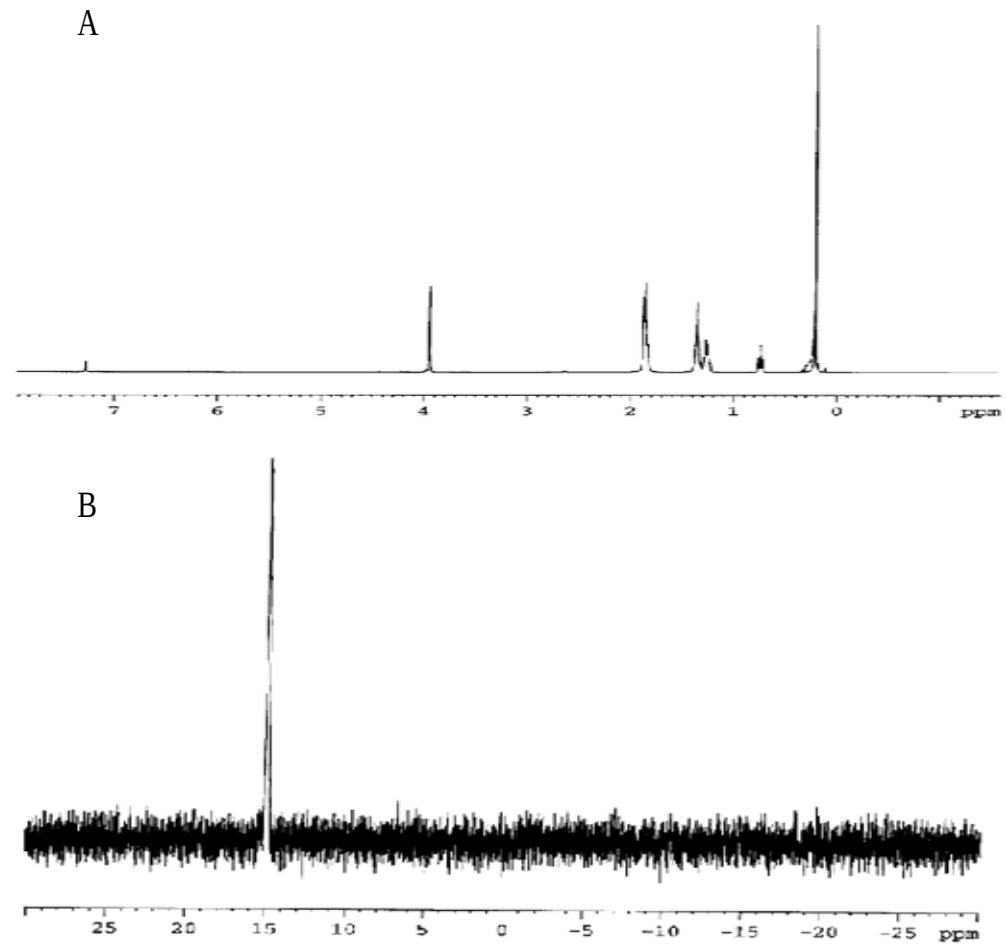
A-15. NMR spectra of pentyldimethylsilanol, A is H-1 NMR, B is Si-29 NMR in Benzene-D_6 solvent



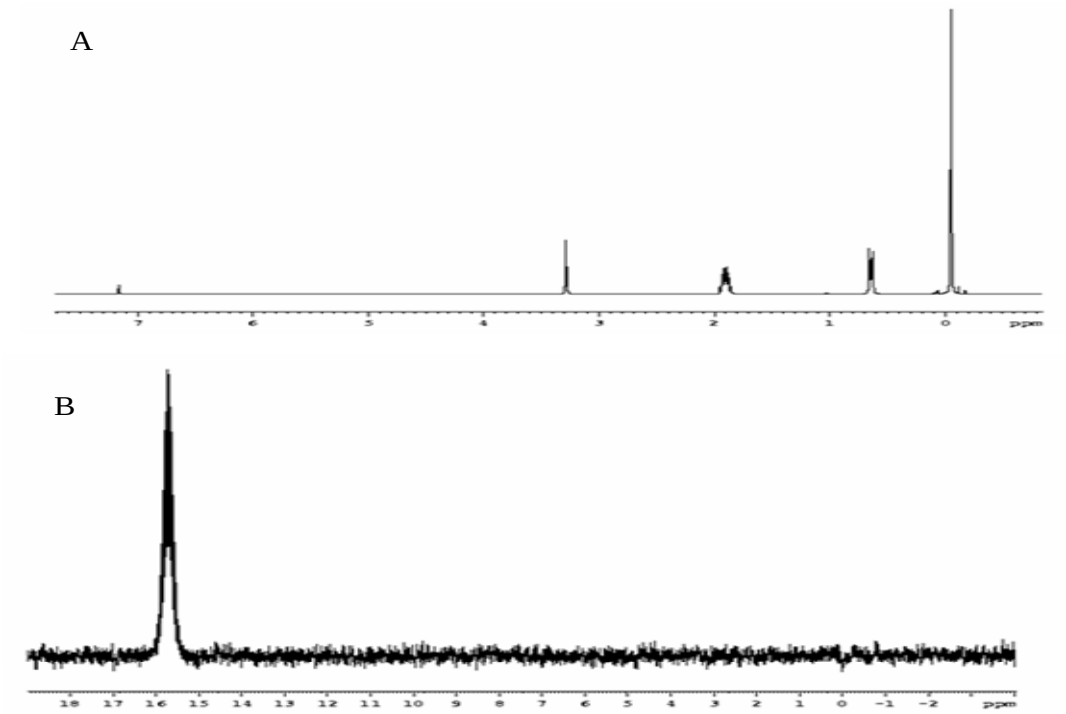
A-16. NMR spectra of hexyldimethylsilanol, A is H-1 NMR, B is Si-29 NMR in Benzene-D_6 solvent.



A-17. NMR spectra of octyldimethylsilanol, H-1 NMR in Benzene-D_6 solvent.



A-18. NMR spectra of cyclohexyldimethylsilanol, A is H-1 NMR, B is Si-29 NMR in Benzene-D_6 solvent.



A-19. NMR spectra of 3,3,3-trifluoropropyldimethylsilanol, A is H-1 NMR, B is Si-29 NMR in Benzene-D_6 solvent.

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BIOGRAPHICAL SKETCH

Yun-Mi Kim was born on April 20, 1974, and raised in Janghung, Chulanamdo, South Korea. She achieved her Bachelor of Engineering in the Department of Chemical Engineering from Dankook University in Seoul, South Korea, in February 1997. She started her professional career at Kukdo Chemical Ltd. as a research engineer in March 1997. Her area of research was development of epoxy resins and curing agents. In July, 2000 she decided to go back to school for an advanced degree. She began studying for the degree of Doctor of Philosophy in materials science and engineering at the University of Florida, Gainesville, in August 2002. In her first year she was involved with research regarding decontamination of tritium. Her specialty areas are polymer science and biomaterials for medical and industry application. In August 2005 she received the degree of Master of Science and continued for her Ph.D. During her graduate study, she published 2 papers and applied for two patents. US patents and European patents are currently pending.